OUTLINES OF

PHYSIOLOGICAL CHEMISTRY

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PREFACE.

THE subject of physiological chemistry is becoming of greater importance every day, but so far as we are aware there is no book of small compass which attempts to deal with the theoretical side of the many questions involved. There are plenty of laboratory guides but in order to grasp the significance of his laboratory work the student has to pick up most of his ideas from books which deal primarily with subjects of purely chemical interest, and only secondarily with those of special importance to the physiologist.

We have endeavored to go straight to the point, dealing only with questions bearing directly on physiological problems.

In order to keep the book within reasonable bounds it has been found necessary to assume for the reader some knowledge of inorganic chemistry, and for the same reason it is impossible to avoid making some general statements which would have to be qualified or modified to some extent if taken up in detail. It is sincerely hoped, however, that no actual errors have been committed. Since this is not intended for a laboratory guide, sufficient details for applying various tests have not been given in most cases. The object has simply been to explain the nature of the reactions.

LOOMIS LABORATORY, NEW YORK,
April, 1904.

OUTLINES OF PHYSIOLOGICAL CHEMISTRY.

CHAPTER I.

THEORY OF SOLUTIONS. IONIZATION.

THE researches of the last fifteen years have made plainer the mechanism of reactions taking place in solution. Practically all of the reactions that are of interest to the physiological chemist take place in solution, so that it is important to know something about the new chemistry.

DISSOCIATION.

When acids, bases and salts are dissolved in water they are said to dissociate, *i. e.*, the molecules to a certain extent break apart. These fragments carry a charge of electricity and are called ions. Thus in a 10 per cent. solution of sodium chloride ($NaCl$) we have $H_2O + NaCl + Na^+ + Cl^-$. Some of the salt exists in the molecular ($Na - Cl$) condition, the remainder being ionized. The metal carries the positive charge and is called the *cation* (*κάτα*, down); the acid (Cl) carries the negative charge and is called the *anion* (*ἄνα*, up).

In an ordinary solution the electrically charged cations and anions are equally distributed throughout, but on passing an electric current through the solution, the cations fly to the cathode or negative $^-$ pole, and the anions to the anode or positive $^+$ pole.

Diagrammatically:

$$\underset{\text{Pole}}{+} \longleftarrow Cl^- \qquad Na^+ \longrightarrow \underset{\text{Pole}}{-}$$

Sulphuric acid (H_2SO_4) added to a solution of barium chloride ($BaCl_2$)

$$2\,H^+ + \boxed{SO_4^- + Ba^+} + 2\,Cl^- \longrightarrow BaSO_4.$$

In this case as fast as the barium ions are neutralized by the sulphate ions, insoluble $BaSO_4$ is formed which does not dissociate and consequently the barium and sulphate ions are gradually removed from the solution.

Dissociation of Acids and Bases.

1. Acids yield H^+ hydrogen ions.

2. Bases yield OH^- *hydroxyl ions.*

1. *Acids* are substances which give a hydrogen ion when dissociated.

Examples:

$H^+ + Cl^-$	Hydrochloric acid.
$H^+ + NO_3^-$	Nitric acid.

It was formerly supposed that acid properties were due entirely to the presence of oxygen. The word means "acid former." We now know that the cause of acidity is the hydrogen ion. The strength of an acid depends entirely upon the number of hydrogen ions present in a solution of it, *i. e.*, upon the extent to which the acid is dissociated.

Taking the sulphur acids:

H_2S	Hydrosulphuric.
H_2SO_3	Sulphurous acid.
H_2SO_4	Sulphuric acid.

We know very well that the one containing the most oxygen is the strongest acid, *i. e.*, the introduction of oxygen facilitates the dissociation of H from the molecule.

On the other hand with the acids of phosphorus

H_3PO_2	Hypophosphorous acid,
H_3PO_3	Phosphorous acid,
H_3PO_4	Phosphoric acid,

the introduction of oxygen decreases acidity, *i. e.*, phosphoric acid is less dissociated than hypophosphorous acid. In a majority of cases the introduction of oxygen facilitates dissociation and therefore increases acidity but the rule does not always hold good.

NOMENCLATURE OF ACIDS.

The method of naming inorganic acids is intended to show the oxygen relations and was adopted when the acid properties were supposed to depend upon the oxygen content.

Example. Acids of Chlorine. Acids containing no oxygen have the prefix hydro.

HCl	*Hydro*chloric.
HClO	*Hypo*chlorous.
$HClO_2$	Chlor*ous*.
$HClO_3$	Chlor*ic*.
$HClO_4$	*Per*chloric.

From these acids the following potassium salts may be formed.

KCl	Potassium *hydro*chloride commonly called chloride.
KClO	Potassium *hypo*chlorite.
$KClO_2$	Potassium chlor*ite*.
$KClO_3$	Potassium chlor*ate*.
$KClO_4$	Potassium *per*chlorate.

Acids ending in *ic* form salts ending in *ate*.

Acids ending in *ous* form salts ending in *ite*.

2. *Bases* are substances which yield hydroxyl (OH) ions on dissociation. The greater the number of hydroxyl ions the stronger the base.

The hydroxides of the alkali metals potassium, sodium, lithium, being very soluble, dissociate readily and are the strongest bases. The hydroxides of the alkaline earths, barium, calcium, strontium, dissociate less readily, so are somewhat weaker bases.

The hydroxides of the heavy metals are but slightly soluble, so are very much weaker. Some of them, like aluminic hydroxide, dissociate in the presence of a strong acid, yielding hydroxyl ions, whilst in the presence of a strong base they yield hydrogen ions. Thus under different conditions they act either as acids or bases, the action being due to the character of their environment. We may have potassium aluminate or aluminum sulphate.

Example of the formation of a salt

$$KOH + HNO_3 = KNO_3 + H_2O$$

$$K^+ + \boxed{OH^- + H^+} + NO_3 \longrightarrow H_2O + K^+ + NO_3^-$$

The latter forming the molecule KNO_3 on concentration of the solution. In representing by equation the reaction between an acid and a base it is therefore always:

$$K\boxed{OH + H}ONO_2 = K - O - NO_2 + H_2O$$

not

$$KO\boxed{H + HO}NO_2 = K - O - NO_2 + H_2O$$

Potassium nitrate, not nitrate of potash.

The above gives a general idea of the mechanism of reactions between inorganic compounds taking place in aqueous solution, and the same applies also to a great extent to organic compounds. But there are a large number of reactions which take

place without dissociation and it is not necessary to refer all of them to the action of + and − ions. Chemical activity does not necessarily depend upon ions.

Chemical Equilibrium.

1. Equilibrium of Reactions.

When two substances which react chemically are brought together in the same solution the reaction begins at once with a certain velocity, which depends upon the temperature of the solution and its concentration. As the reaction proceeds its speed gradually decreases until the products of the action have attained a definite concentration. This is a condition of equilibrium and no matter how long the substances are left in the solution there is no further change in the concentration.

This is generally illustrated by the action of acetic acid upon alcohol.

Alcohol + acetic acid = acetic ester + water.

The acid begins to act upon the alcohol at once and as it is gradually used up the amount of ester formed in a unit of time decreases until finally we have a condition where the alcohol and acid do not further decrease, and the ester and water do not increase.

This is a condition of equilibrium. There is still some free acid and some free alcohol and these two substances continue to react on one another, yet the amount of each in the solution does not decrease. This is explained by the fact that this reaction, like chemical reactions in general, is a reversible one.

Reversion.—When acetic ester is added to water it reacts with the latter to form alcohol and acetic acid. A simple way of writing the reaction is:

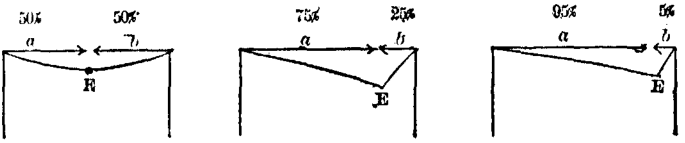
Alcohol + acetic acid $\rightleftarrows$ acetic ester + water.

The double arrows indicate that the reaction may proceed in either direction.

In the first reaction described, as soon as some ester is formed it begins to react with the water to form alcohol and acetic acid, *i. e.*, to reverse the action. The velocity of the reverse action is slow at first because the concentration of the ester is small, but it increases with the latter until finally the amount of ester formed in a unit of time is equal to the amount decomposed. The condition of equilibrium is then the condition when the velocities in the two opposite reactions are equal.

There is a condition of equilibrium for every reaction, but in some cases the action in one direction is so enormously stronger than the action in the other direction, that the former is able to push the latter over almost to a vanishing point, and consequently the reaction may appear to be practically complete.

The point of equilibrium may be given diagrammatically for three imaginary different reactions. The length of the arrows indicates the vigor in each direction.



E point of equilibrium.

This method of considering chemical reactions is of great value to the physiological chemist, and may be approached from another point of view.

2. Equilibrium of Solutions.

Suppose we make what is called a saturated solution of sodium chloride. The conditions are as follows:

$$\underset{\substack{\text{Solid.}\\1}}{NaCl} \rightleftarrows \underset{\substack{\text{In solution.}\\2}}{NaCl} \rightleftarrows \underset{\substack{\text{Ionized.}\\3}}{Na^+ + Cl^-}.$$

When a solution is saturated as much solid dissolves as precipitates out in a given time. There is therefore equilibrium between 1 and 2. There is also equilibrium between 2 and 3 when the amount which dissociates equals the amount returning to the undissociated condition. There are here two opposing actions. Some of the molecules of salt are breaking apart into ions, whilst some of the ions are combining to form molecules of salt.

If we now pass into this saturated solution some gaseous hydrochloric acid, some of it dissolves and dissociates. We thereby increase the number of Cl ions.

$$HCl \rightleftarrows H^{+} + Cl^{-}.$$

Since now the Na^{+} ions come in contact with Cl^{-} ions more often than they previously did, the combining action will exceed the dissociation for a time, and more salt will pass into the undissociated condition. But the solution is already saturated with undissociated salt, so that some must precipitate out in solid form and this precipitation will continue until a new plane of equilibrium is established.

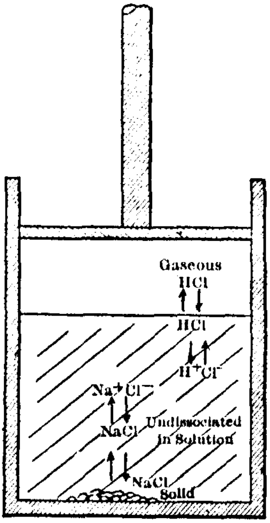


FIG. 1.

Suppose we have the conditions represented in the drawing. A solution saturated with both hydrochloric acid and sodium chloride and arranged so that pressure can be produced upon the gaseous HCl above the solution. The conditions of equilibrium may be thus represented:

$$NaCl \rightleftarrows NaCl \rightleftarrows Na^{+} + Cl^{-} + Cl^{-} + H^{+} \rightleftarrows HCl \rightleftarrows HCl$$

NaCl	NaCl	$Na^{+}+Cl^{-}+Cl^{-}+H^{+}$	HCl	HCl
Solid undissolved.	In solution undissociated.	Ions.	In solution undissociated.	Gas above liquid.

There is complete equilibrium.

1. As much salt dissolves in a unit of time as precipitates from solution.

2. As much salt dissociates into ions in a unit of time as is formed by recombination of ions.

3. As many HCl molecules fly off from the surface of the liquid in a unit of time as there are molecules entering the liquid from the gas.

We now bring the piston down, thereby increasing the pressure on the HCl gas. This causes more gas to dissolve, which in turn increases the number of H and Cl ions. The increase in Cl ions causes an increased formation of undissociated sodium chloride which in turn causes a separation of a small amount of solid salt from the solution.

We might cause further changes by altering the temperature.

There is everywhere a continual striving for equilibrium which causes chemical changes and physical changes as well. The factors concerned in the equilibrium of living matter are so numerous and complex that very slight alteration in the system of reactions which make life possible may cause serious results. Complete universal equilibrium is the end towards which we are moving.

Catalysis. — In the example given above acetic ester in the presence of water decomposes with the formation of alcohol and acetic acid. The rate of decomposition is slow, but if a small quantity of some other acid is added the reaction goes on much faster. The acid added does not apparently enter into the reaction itself, but hastens it to a remarkable degree. The more concentrated the acid the greater the velocity of the reaction. Strong acids have a greater effect than weak acids and experiments have shown that the speed of the reaction is nearly proportional to the concentration of H ions in the solu-

tion. The action of the acid is called catalytic and the acid itself the catalyzer.

Catalyzers do not enter into the action themselves, but a very small quantity of a catalyzer is able to cause a large effect upon the speed of reaction.

The physiological chemist is chiefly interested in the catalytic action of the enzymes or ferments secreted by cells with the object of accelerating reactions which are of use to themselves.

Colloids and Crystalloids.—Soluble substances are divided into two groups, the basis for the classification being their behavior in regard to diffusion.

1. *Crystalloids* are those which have relatively small molecules and readily diffuse through a membrane such as parchment.

2. *Colloids* possess molecules too large to pass readily through pores of a membrane, so diffuse very slowly or not at all. Proteid molecules for instance.

In many cases, however, it is not the actual size of the molecules which prevents diffusion, but the fact that the molecules cling together to form what are called *solution aggregates.*

Colloidal Solutions of Metals.—It has been found possible within the last few years to obtain some of the metals, gold, silver, platinum, in the condition of a colloidal solution.

If two platinum wires carrying a strong electric current are brought close together below the surface of water an electric arc will be formed. Very fine pieces of platinum are torn off from the cathode (negative pole) and the solution soon has a dark brown color. No pieces of platinum can be seen in the liquid even under the highest powers of the microscope. They

exist however as *solution aggregates*, not as individual molecules.

Such metallic colloidal solutions are *catalyzers* and have certain similarities to enzymes.

1. A very small quantity of metal in this condition can cause a great change in the speed of the reaction.

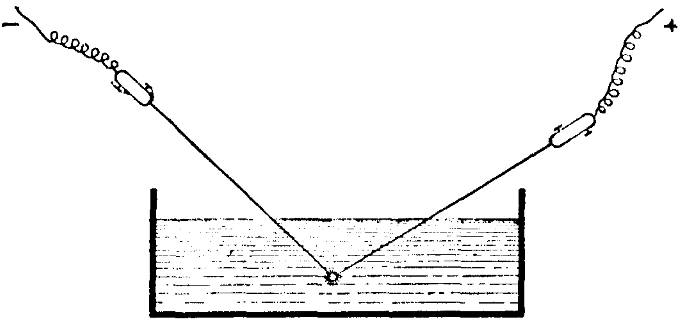


FIG. 2.

2. Certain poisons which destroy enzymes have an analogous action on these colloidal solutions.

AGGREGATION, SUSPENSION, AND PRECIPITATION.

The solution aggregates being minute collections of molecules are simply small lumps of the substance. In various ways, by the addition of salts for instance, the aggregates can be increased in size so that several cling together. More and more aggregates attach themselves to the larger lumps until these become visible, at first under the microscope and then to the eye, forming a "*suspension*." Finally the lumps become so large that they sink and the colloid is "*precipitated*." There is then no real difference between aggregation, suspension and precipitation. They are only different stages of a process.

Oxidation and Reduction.

1. *Oxidation* is normally the addition of oxygen to some substance.

Reduction is the taking away of oxygen from some substance.

Oxidizing agents are substances containing a large amount of oxygen which is easily set free, *i. e.*, it is in loose combination.

Reducing agents are substances which have a strong affinity for oxygen and combine with it readily.

The above definitions are not sufficient to cover the case. It is a general rule that reduction and oxidation go on simultaneously but in different substances.

$$CuO + H_2 = Cu + H_2O.$$

The copper is reduced, the hydrogen oxidized.

Copper oxide is the oxidizing agent, hydrogen the reducing agent.

It is obvious that oxidation and reduction go on simultaneously in cases where oxygen is taken from one compound by another. One is oxidized, the other reduced.

2. If the combining power of a metal or electro-positive substances, *i. e.*, substances behaving like metals, is reduced, this is also called *reduction*.

$FeCl_3$		$FeCl_2$
Ferric chloride	reduced to	ferrous chloride.

In the first instance the combining power of Fe is with 3 Cl atoms, in the second it only combines with 2 Cl atoms.

The reverse of this is called *oxidation* for convenience although no oxygen is added.

$FeCl_2$		$FeCl_3$
Ferrous chloride	oxidized to	ferric chloride.

3. If an electro-negative radical is taken away from an electro-positive radical the latter is *reduced*.

Cobalt (Co^{+}) is a positive radical, being a metal.

Chlorine (Cl^{-}) is a negative radical.

$$Co \begin{cases} Cl + H \\ Cl + H \end{cases} \rightarrow 2HCl$$

The cobalt is reduced, the negative radical being removed.

The reverse of this is also called *oxidation* for convenience

$$Co + Cl_2 = Co \begin{cases} Cl \\ Cl \end{cases}$$

The cobalt is oxidized to $CoCl_2$.

In the first case the hydrogen is *oxidized* to HCl.

Hydrogen is a reducer. — At the moment of being freed from combination it is said to be nascent and is then a much more active reducer, *i. e.*, combines more readily, than when in the gaseous condition. In the gaseous condition it exists as a molecule and may be represented by the formula H — H or H_2 whilst nascent hydrogen is in the atomic form H^{-}.

We may pass gaseous hydrogen through a solution of ferric chloride for a long time without reducing it to the ferrous state, but on adding acid and then some zinc to the solution, ($H_2SO_4 + Zn = ZnSO_4 + H + H$), hydrogen is set free in the nascent condition and the ferric chloride quickly reduced:

$$FeCl_3 + H = FeCl_2 + HCl.$$

A very ready means for obtaining nascent hydrogen in aqueous solutions is by the use of sodium or sodium amalgam.

$$Na + H_2O = NaOH + H.$$

Oxygen. — The oxygen of the atmosphere is in the molecular condition O = O or O_2.

Nascent oxygen may be obtained by the decomposition of hydrogen peroxide, $H_2O_2 = H_2O + O$, or by the decomposition of water by chlorine, $H_2O + Cl_2 = 2HCl + O$.

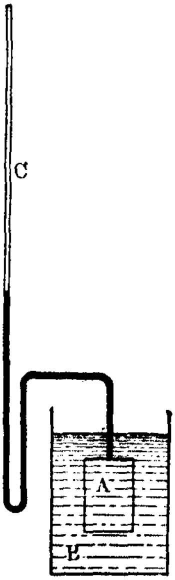
Ozone is an allotropic form of oxygen represented by the formula

It decomposes very readily, yielding nascent oxygen and is therefore a very active oxidizing agent.

Allotropism is a term used to denote the existence of the same element in different physical forms. These forms are probably due to a different atomic arrangement in the molecule. Carbon, sulphur, phosphorus and oxygen are prominent elements in living matter and have allotropic forms.

OSMOTIC PRESSURE.

Matter in the gaseous form is in much the same condition as when in a solution. In both cases the molecules are free to move about, and in both cases the movement expresses itself as a pressure. The pressure exerted by a gas is familiar to all. The analogous pressure of the substance in solution, although more difficult to measure, obeys the same laws. It is called the osmotic pressure.



A Porous cup containing sugar solution
B Jar of water
C Pressure tube

FIG. 3.

This was first demonstrated by filling a bladder with alcohol and then immersing it in water. The water can pass in, but the alcohol cannot pass out

to any extent, and the increased pressure will cause the bladder to burst.

The demonstration of osmotic pressure requires a semi-permeable membrane, *i. e.*, a membrane permeable to the solvent, but not to the dissolved substance.

Such a membrane may be prepared by precipitating copper ferrocyanide in an unglazed earthenware cup. If such a cup is filled with a sugar solution, attached to a manometer and then immersed in a jar of water the manometer will soon indicate a rise of pressure in the cup. This pressure will continue to increase until the final pressure will be the same as it would be if the dissolved substance were a gas and occupied the same volume as the sugar solution.

The pressure attained depends upon the concentration and temperature of the solution. The explanation of gas pressure in terms of the kinetic theory is that it is due to a bombardment of the walls of the confining vessel with particles of gas but there is no satisfactory explanation of the cause of osmotic pressure.

The many problems of filtration, secretion and absorption in the body have caused physiologists to be very much interested in the phenomena of osmotic pressure.

Calculations of a Formula.

To calculate the formula of a compound two things must be known:

1. The molecular weight.
2. The percentage composition.

The molecular weight may be determined by:

1. Measuring the elevation in boiling point or depression in freezing point caused by dissolving a known amount of the compound in a known amount of liquid.

2. By determining its vapor density, providing it will volatilize without decomposition.

3. Other methods which cannot be described.

The percentage composition is determined by ultimate analysis of a pure sample of the compound.

Suppose we have a compound with a molecular weight 180, percentage composition

C — 40	per cent.	
H — 6.66	"	
O — 53.3	"	

The percentage of each element is divided by the atomic weight of that element.

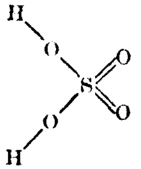
C — 40/12	— 3.33	1
H — 6.66/1	— 6.66	2
O — 533/16	— 3.33	1

Express the ratio in whole numbers and the simplest formula for the given substance would be CH_2O which would have a molecular weight of 30. The compound in question had a molecular weight of 180 so it must be six times as large as the simpler one or $C_6H_{12}O_6$.

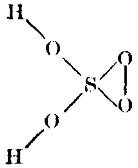
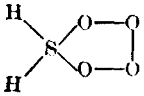
The difficulty of determining the molecular weight of many substances prevents an empirical formula from being exactly determined. Two substances may have the same percentage composition and yet have a very different molecular weight, as in the example given above, formaldehyde and dextrose.

The empirical formula of a compound shows the number of atoms of different elements there may be in a molecule but it does not show their arrangement. The behavior of any compound is much more intelligible if we know how the different atoms are combined. This atomic arrangement is shown by the graphic formula:

H_2SO_4 empirical formula.

(1)  graphic formula.

There are obviously other ways of arranging the atoms in this case,

(2)  or (3) 

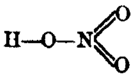
Sulphur may have a valence of 4 or 6, oxygen always being 2, hydrogen 1. There are many reasons why formula 1 is to be regarded as correct. In the first place compounds having oxygen combined with itself are very unstable

$$H_2O_2, \quad H - O - O - H,$$

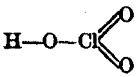
or O_3 ozone

Sulphuric acid is very stable, therefore it cannot be either (2) or (3).

For the same reasons HNO_3 is written



and $HClO_3$,

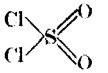


Cl when oxidized may have 1, 3, or 5 valences.

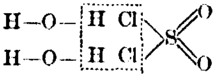
There are reasons also for believing that the hydrogen is tied to the sulphur by means of oxygen.

SO_2,

combines with chlorine to form a compound



this reacts with water to form sulphuric acid.



Reasons Why Reactions Take Place.

An old rule which applies to a large number of cases is the following: "Whenever an insoluble or volatile substance can be formed, it will be formed to the full extent of the components." As an example we may consider the decomposition of calcium carbonate by heat:

$$CaCO_3 \rightleftarrows CaO + CO_2.$$

CO_2 is volatile and as long as there is no hindrance to its escape the decomposition continues. If, however, the calcium carbonate is heated in a strong closed steel cylinder so that the CO_2 cannot escape, the calcium carbonate is found unchanged when the cylinder is opened after cooling.

The reactions which are used in ultimate analysis obey this law. In determining the amount of sulphur in organic substances the sulphur is oxidized to a sulphate which is precipitated by some soluble salt of barium, such as the chloride.

$$Na_2SO_4 + BaCl_2 \rightleftarrows 2\,NaCl + BaSO_4$$

$$2\,Na^+ + SO_4^- + Ba^{++} + 2\,Cl^- \rightleftarrows BaSO_4 - 2\,Na^+ + 2\,Cl^-.$$

The $BaSO_4$ is almost completely insoluble and is therefore removed from the solution. By adding an excess of barium all the sulphate will be precipitated. The reaction will run to an end practically.

This is a case where the equilibrium is at vanishing point.

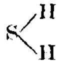
A few graphic formulas of inorganic compounds which will be of use are given. They will serve also as an introduction to the numerous graphic formulas of organic compounds with which we shall have to deal.

Fe, 2 or 4 valences, Cl, 1 valence.

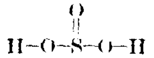
Ferric chloride, Fe_2Cl_6, usually written $FeCl_3$. Basis for *ferri* compounds.

Ferrous chloride, $FeCl_2$. Basis for *ferro* compounds.

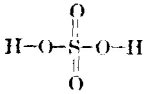
S, 2, 4 or 6 valences, H, 1 valence, O, 2 valences.



Hydrosulphuric acid.

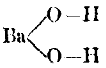


Sulphurous acid.

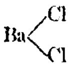


Sulphuric acid.

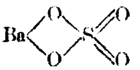
Ba, 2 valences, *Ca*, 2 valences.



Barium hydroxide.



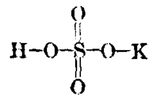
Barium chloride.



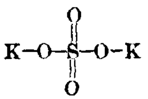
Barium sulphate.

Calcium (Ca) combines in the same way.

K, 1 valence, Na, 1 valence.



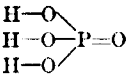
Monopotassic sulphate, $KHSO_4$



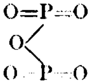
Dipotassic sulphate, K_2SO_4

Na combines in the same way.

P, 3 or 5 valences.



Phosphoric acid, H_3PO_4

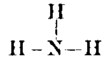


Phosphoric anhydride, P_2O_5

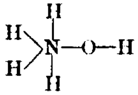
Phosphoric anhydride is two molecules of H_3PO_4 from which three molecules of H_2O have been driven off.

$$P_2O_5 + 3H_2O = 2H_3PO_4$$

N, 3 or 5 valences.



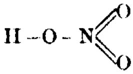
Ammonia gas, NH_3



Ammonium hydroxide, $NH_4OH(OH^-$ ion)

H – O – N = O

Nitrous acid, $HNO_2(H^+$ ion)



Nitric acid, $HNO_3(H^+$ ion).

Ultimate Analysis.

The determination of the elementary content of any substance is called ultimate analysis.

The elements which occur in proteid and are therefore of most interest to us are C, O, H, P, S and N.

The first three of these are estimated in various ways, but hardly concern us since it is only exceptionally that determinations of C, O and H content of proteid are made. P, S and N estimations however are constantly required.

P Content. — The P in the substance is oxidized to phosphoric acid and precipitated as insoluble magnesium phosphate and the amount of P calculated. In the words of the chemist it is estimated as P_2O_5.

S Content. — The sulphur is completely oxidized to sulphuric acid or soluble sulphates, then precipitated as the insoluble $BaSO_4$, which is weighed and the amount of S calculated. Estimated as barium sulphate.

N Content. — N is estimated as NH_3 by Kjeldahl's method. (see text-books).

CHAPTER II.

ORGANIC CHEMISTRY OR THE CHEMISTRY OF CARBON COMPOUNDS.

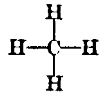
THE element carbon differs from other elements in its remarkable power of combining with itself to form stable compounds. Its combining power or valence is determined by an analysis of many of its simplest compounds to be 4, *i. e.*, it can combine with four atoms of hydrogen.

If we consider the structure of the compounds of hydrogen and oxygen, H_2O and H_2O_2 we see a reason for the difference in properties due to the fact that oxygen is combined with itself. H — O — H is one of the most stable compounds known. H — O — O — H is very readily decomposed, the element of weakness being the tie between the O atoms.

Again the diazo compounds which contain the atom group — N = N — are very explosive, *i. e.*, the compound is unstable, the two N atoms being easily separated. But carbon may combine with itself (C — C — C — C) to an almost unlimited extent, and still form stable compounds.

It is this property of the C atom which makes possible the immense and complicated molecules of organic matter.

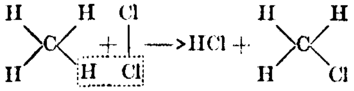
The simplest compounds of carbon are the hydrocarbons, substances which contain only carbon and hydrogen. The simplest of the hydrocarbons is represented by the formula CH_4. Because of the large number of hydrocarbons and their great variation in structure it is necessary to arrange the atoms graphically in order to grasp the full significance of their construction. The graphic formula of CH_4 may be thus written



Methane (marsh gas).

Methane is the simplest of a series of compounds called the methane or marsh gas series of hydrocarbons.

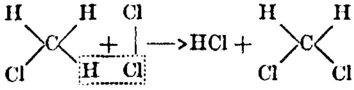
The H atoms may be replaced by other elements, forming what are called substitution products. For instance if chlorine is mixed with methane in equal volume and exposed to sunlight, a reaction which may be represented as follows, takes place.



Methane + chlorine = hydrochloric acid + monochlormethane.

$$CH_4 + Cl_2 \quad = HCl \qquad + CH_3Cl$$

On increasing the amount of chlorine the change may go further



Monochlormethane. Dichlormethane.

$$CH_3Cl + Cl_2 = HCl + CH_2Cl_2$$

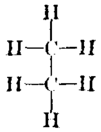
By continued action a series of chlorine substitution products of methane are formed.

CH_3Cl	Monochlormethane,
CH_2Cl_2	Dichlormethane,
$CHCl_3$	Trichlormethane,
CCl_4	Tetrachlormethane.

In the same way iodine and bromine substitution products may be formed.

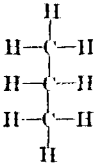
These compounds are said to be saturated because the maximum combining power of the C atom is used.

The next in this series of hydrocarbons contains two atoms of carbon held together by a single bond, the three remaining valences of each C atom being brought to saturation by hydrogen. The only possible way of representing this graphically is by the formula



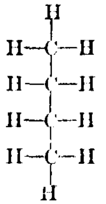
Ethane, C_2H_6.

Ethane is a gas having much the same chemical behavior as methane. We may consider it as a substitution product of methane, formed by substituting a methyl group, CH_3, for one of its H atoms. In the same way a methyl group if substituted in ethane, gives propane, the next higher compound of the series.

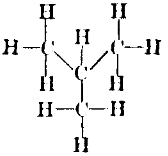


Propane, C_3H_8 or $CH_3.CH_2.CH_3$.

It is evident from the graphic formula of propane that the central C group differs from the other two, and therefore, on forming the next higher compound of the series it will make a difference whether we substitute the methyl group on one of the C atoms at the end of the chain or the central one. We may thus form two hydrocarbons having the formula C_4H_{10}(butane), but differing in the arrangement of the groups.



Normal butane, C_4H_{10}.



Iso-butane, C_4H_{10}.

The two compounds, although they have precisely the same amount of C and H, present different characteristics — reactions, appearance, odor, etc. — which can only be explained on the basis of differing internal arrangement.

Such compounds are said to be *isomers* and as we go higher in the series the number of possible arrangements and consequently the number of isomers increases with alarming rapidity.

Methane	CH_4	— 1	Pentane	C_5H_{12}	— 3	Octane	C_8H_{18}	— 18
Ethane	C_2H_6	— 1	Hexane	C_6H_{14}	— 5	Nonane	C_9H_{20}	— 35
Propane	C_3H_8	— 1	Heptane	C_7H_{16}	— 8	Decane	$C_{10}H_{22}$	— 75
Butane	C_4H_{10}	— 2						

A common difference CH_2 exists between the successive members so that a general formula for the series may be given as C_nH_{2n+2}.

The hydrocarbons are such inactive bodies chemically that they are of no particular interest to us except to take as simple examples with a view to gaining some elementary ideas of the structure of organic compounds. The lower (those with a few C atoms) members of the series are gases, and as the molecular weight increases the boiling point rises; the higher members being the wax-like bodies called paraffins.

Such a series as that given above in which there is a similarity in constitution but a gradual and regularly increasing variation in properties is said to be homologous, and the members are called homologues.

Example: Butane, C_4H_{10}, is a higher homologue of propane, C_3H_8.

There are many such series among the organic compounds.

By oxidation of the hydrocarbons a number of interesting and well-known compounds, the alcohols, aldehydes and organic acids are obtained.

We can now trace in regular sequence the processes of oxidation and the compounds resulting from it.

ALCOHOLS, ALDEHYDES, ACIDS.

1. *Alcohols.* — If methane is completely oxidized the result is the formation of carbon dioxide and water.

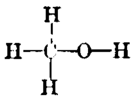
$$CH_4 + 2O_2 \rightarrow CO_2 + H_2O.$$

If the oxidation is carefully regulated however the intermediate products in the process may be obtained. We may thus introduce one O atom into the methane molecule

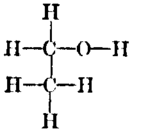
In this new substance the H atom which is tied to the carbon through the oxygen atom behaves very differently from the remaining three, being much more easily split off, thus allowing the O to attach itself to other compounds. In other words the new compound is chemically active whilst the hydrocarbons are chemically inert.

The substance CH_3OH is the simplest of a new homologous series of compounds obtained by partially oxidizing the hydrocarbons. These are the alcohols, the one already given being methanol (methyl alcohol), commonly called wood alcohol because it is obtained by the dry distillation of wood.

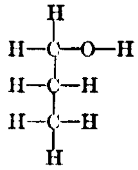
The alcohols are always designated by the suffix "ol."



Methanol (methyl alcohol), CH_3OH.



Ethanol (ethyl alcohol), C_2H_5OH.



Propanol (propyl alcohol), C_3H_7OH.

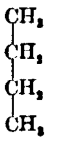
Butanol and higher homologues.

General formula $C_nH_{2n+1}OH$.

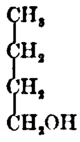
The alcohol of everyday use is ethanol or ethyl alcohol obtained as a product of fermentation.

The alcohols exist in isomeric forms but since two or more isomeric alcohols may be derived from one hydrocarbon the number of isomers is vastly greater. Fortunately only a few are of importance for us.

Alcohols are divided into three classes, primary, secondary and tertiary, the basis of the classification being the arrangement of the molecule. This may be illustrated by the butyl alcohols C_4H_9OH.

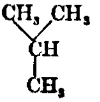


Normal butane, C_4H_{10}.

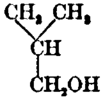


Primary normal butanol (primary butyl alcohol), C_4H_9OH.

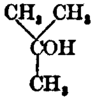
Secondary normal butanol (secondary butyl alcohol), C_4H_9OH.



Iso butane, C_4H_{10}.



Primary iso butanol (primary butyl-alcohol), C_4H_9OH.



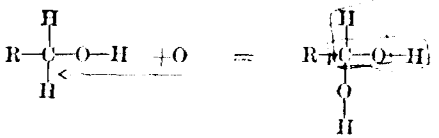
Tertiary butanol (tertiary butyl-alcohol), C_4H_9OH.

GROUPS CHARACTERISTIC OF ALCOHOLS.

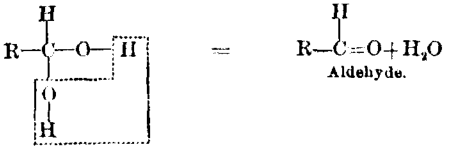
(1)	$R—CH_2OH$	Primary	R being any organic radical.
(2)	$R_2{>}CHOH$	Secondary.	
(3)	$R_3{>}COH$	Tertiary.	

2. *Aldehydes, Ketones.*—The alcohols have different characteristics, but the differences in their oxidation products is more striking.

A. *Primary alcohols* on further oxidation yield aldehydes.

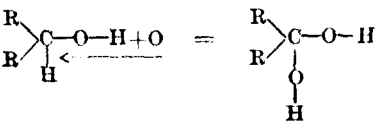


But since the C atom can never support more than one chemically active (OH) group, this combination is an impossibility, so something must happen. H_2O is immediately thrown off and the C attaches itself to the remaining O by two valences.

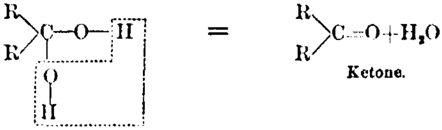


The group CHO is characteristic of aldehydes.

B. *Secondary alcohols,* on oxidation yield ketones.



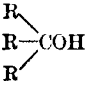
Again H_2O is thrown off and we get



Ketone.

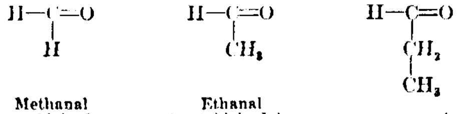
The group CO is characteristic of ketones.

Since the *tertiary alcohols* have the formula



there is obviously no H left for oxidation. If the COH group is further oxidized to C = O, the C atom must take a valence from one of the radicals, so that the compound would break up.

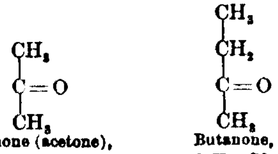
The aldehydes are always designated by the suffix "al." Thus we have:



Methanal (formaldehyde), HCHO. Ethanal (acetaldehyde), $CH_3.CHO$. Propanal (propionic aldehyde), $C_2H_5.CHO$. Butanal and higher homologues.

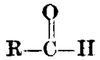
Formalin is the commercial formaldehyde 40 per cent. in water.

The Ketones obviously cannot exist unless the chain contains three or more C atoms. They always have the suffix "one."



Propanone (acetone), $C_2H_6.CO$. Butanone, $C_3H_8.CO$. Pentanone and higher homologues.

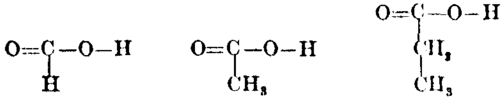
3. *Acids.*—The *aldehydes*



have one H left which can be oxidized to form an acid.

Aldehyde. Acid.

Acids always have the suffix acid so we have—

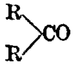


Methanacid (formic acid), HCOOH. Ethanacid (acetic acid), $CH_3 . COOH$. Propanacid (propionic acid), $C_2H_5 . COOH$. Butanacid (butyric acid) and higher homologues.

General formula $C_nH_{2n}O_2$.

The group COOH is characteristic of the acids.

The Ketones



have no H left for oxidation. On further oxidation the compound breaks up. Summarizing

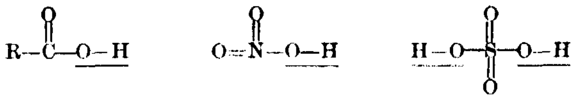
On oxidation.

Primary alcohols → Aldehydes → Acids → Break up.
Secondary alcohols → Ketones → Break up.
Tertiary alcohols → Break up.

Acids then can only be derived from primary alcohols, (CH_2OH) the series so formed being called the *Fatty acid series,* since certain of the higher homologues enter into the composition of the fats.

The members of the fatty acids series are *monobasic,* since there is only one OH group to enter into combination.

A comparison may be made with the mineral acids.



Fatty acid monobasic. Nitric acid monobasic. Sulphuric acid dibasic.

The following table summarizes the four homologous series so far discussed.

	Chemically Inert.	Chemically Active. Basic.	Chemically Active. Neutral.	Chemically Active. Acid.
General Prefix.	Hydro-carbons.	Alcohols. Suffix "ol."	Aldehydes. Suffix "al."	Acids. Suffix "acid."
Meth.	CH_4	CH_3OH	$H.CHO$	$H.COOH$
Eth.	C_2H_6	C_2H_5OH	$CH_3.CHO$	$CH_3.COOH$
Prop.	C_3H_8	C_3H_7OH	$C_2H_5.CHO$	$C_2H_5.COOH$
But.	C_4H_{10}	C_4H_9OH	$C_3H_7.CHO$	$C_3H_7.COOH$

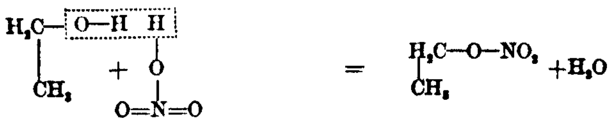
and so on for the higher homologues, which are designated by the number of C atoms they contain — Pent., Hex., Hept., etc.

A study of the table brings out some interesting points.

1. The introduction of O changes chemically inert into chemically active compounds. This rule is not universal but holds good for *many* organic compounds.

2. The introduction of a *little* O produces basic alcohols which will readily unite with acids:

Example:



Ethyl alcohol + Nitric acid = Ethyl nitrate + water.

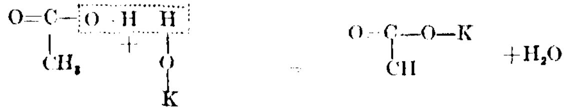
The alcohols of this series have only one active OH group, so are called *monoatomic*.

3. The introduction of a *little more* O produces neutral aldehydes, which have no special affinity for either acids or bases, but being chemically active afford some very interesting reactions with various organic compounds.

These reactions will have to be discussed later since they occur with complex substances, the constitution of which has not yet been considered. The reaction with Fehling's solution, however, will be explained directly.

4. The introduction of a *further amount* of O produces acids which will readily combine with a base.

Example:



Acetic acid + Potassium hydroxide = Potassium acetate + water
$CH_3 \cdot COOH$ KOH $CH_3 \cdot COOK$

It has been explained in Chapter I. that an increase of O does not necessarily mean an increase of acidity, but with organic compounds this is nearly always the case.

It may however be remarked that the alcohols are weak bases and the fatty acids weak acids.

1. An alcohol in combination with an acid is easily driven out by a strong mineral base such as KOH.

Example:

$$CH_3 \cdot CH_2{-}O{-}NO_2 + KOH = K{-}O{-}NO_2 + CH_3 \cdot CH_2OH$$

Ethyl nitrate + Potassium hydroxide = Potassium nitrate + Ethyl alcohol.

2. A fatty acid in combination with a base is easily driven off by a strong mineral acid.

Example:

$$CH_3 \cdot COOK + HONO_2 = KONO_2 + CH_3 \cdot COOH$$

Potassium acetate + Nitric acid = Potassium nitrate + Acetic acid.

TESTS.

1. *For Alcohols.* — Iodoform test, see p. 54.

2. *For Aldehydes.* — The aldehydes representing an intermediate stage of oxidation are always eager for more O, and take it up whenever it is offered in an accessible form.

They will therefore seize O from other compounds in cases where the latter carry it loosely. Such a compound is found in cupric hydroxide. Aldehydes therefore are *reducers.* In cases where it is required to deprive certain compounds of their oxygen, or a part of it, the aldehydes are often employed for this purpose.

CHEMISTRY OF FEHLING'S SOLUTION.

Copper has two hydroxides and corresponding insoluble oxides.

Cuprous hydroxide yellow. Cuprous oxide red. Cupric hydroxide blue. Cupric oxide black.

Cupric hydroxide, $Cu(OH)_2$, is blue when first precipitated. On adding alkali (KOH) and boiling, H_2O is driven off and black cupric oxide CuO which is insoluble is formed and falls as a precipitate.

$$Cu(OH)_2 = CuO + H_2O.$$

But if $Cu(OH)_2$ is heated with a reducing agent such as an aldehyde (glucose is an aldehyde), the cupric hydroxide decomposes still further to cuprous oxide.

$$2Cu(OH)_2 = Cu_2O + H_2O + O,$$

the O being taken up by the reducer.

The bright red Cu_2O is insoluble and this is the red precipitate which is looked for on testing solutions for the presence of reducers — glucose, etc.

But unless there is enough reducer to change all the cupric hydroxide into red cuprous oxide, some of it will precipitate as black cupric oxide which masks the red color. To avoid this tartaric acid is used, which forms a soluble colorless compound with the cupric oxide but does not affect the red cuprous oxide.

The point is to add something by which the CuO is made soluble and colorless, but which leaves the Cu_2O unchanged.

Fehling's solution A is $CuSO_4$.

Fehling's solution B is KOH + some tartrate.

On mixing the two the following reaction occurs:

$$CuSO_4 + 2KOH = K_2SO_4 + Cu(OH)_2$$

The $Cu(OH)_2$ must be used fresh as it decomposes slowly in the cold, so the two solutions are not mixed until just before using.

Nylander's Test. — Glucose and other aldehydes reduce other metallic oxides in alkaline solution. The reduction of bismuth oxide is the basis for Nylander's test. In this case the metallic oxide in alkaline solution will keep for months. In boiling with a reducing substance a black deposit of bismuth is formed.

The Ketones (CO) have no spare H for oxidation so are not greedy for O, and do not reduce copper oxide. The reaction with Fehling's solution is therefore an aldehyde, not a ketone reaction.

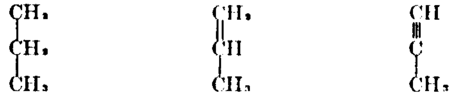
3. *For Acids.* — Treated with $FeCl_3$ the lower (homologues of the) fatty acids and their salts,—formates, acetates, etc., afford a red color due to the formation of colored Fe compounds. The reaction is not well understood and need not be discussed in detail.

Unsaturated Compounds.

In addition to the homologous series already discussed there are others called unsaturated compounds, in which two C atoms

are joined by two or three links instead of one. The hydrocarbons of such series have the suffix ene and ine.

Ethane. Ethene (ethylene gas). Ethine (acetylene gas).



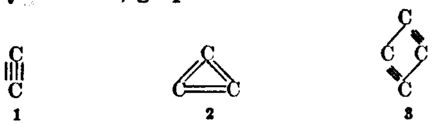
Propane. Propene. Propine.

These likewise form long series of oxidation and substitution products but the compounds formed are of little interest to physiological chemists, who do not deal in gases and mineral oils.

It will be noticed that in these compounds the C atom always has four occupied links, but since in the unsaturated hydrocarbons there are two adjacent carbon atoms united doubly or trebly to each other the ratio of hydrogen to carbon is smaller than in the saturated series.

The Carbon Molecule.

The C atom can attach itself to another or to two others by all of its four valences, thus forming the carbon molecule. Three forms of allotropism are possible which are probably represented by charcoal, graphite and diamond.



1 2 3

Only one grouping is possible with 1, but it is obvious that the rings 2 and 3 can theoretically contain an unlimited number of carbon atoms, but nothing is known of the way in which the carbon atoms hang together to form the molecule.

CHAPTER III.

COMBINATIONS OF THE OXIDATION PRODUCTS OF THE PARAFFINS WITH EACH OTHER.

Ethers, Esters, Anhydrids.

The ethers are the result of a reaction between the OH groups of two alcohols with loss of water; in other words of two bases. Strong mineral bases cannot do this, but weak organic bases are able to.

Example.

$$\begin{array}{lll} \text{Ethanol.} & CH_3\cdot CH_2O\,[H] & \\ & + & = \; \begin{array}{l} CH_3\cdot CH_2 \\ \quad\quad >O + H_2O \\ CH_3\cdot CH_2 \end{array} \\ \text{Ethanol.} & CH_3\cdot CH_2\,[OH] & \end{array}$$

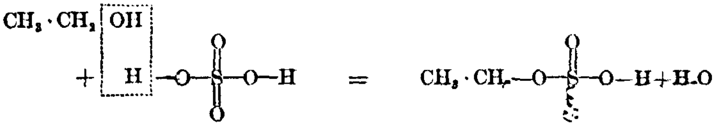
Ethanether (ether).

The alcohols which enter into the combination are not necessarily similar. The ether may be methyl ethyl, or ethyl propyl, etc.

$$\begin{array}{l} CH_3 \\ \quad >O \\ CH_3\cdot CH_2 \end{array} \qquad \begin{array}{l} CH_3\cdot CH_2 \\ \quad\quad >O \\ CH_3\cdot CH_2\cdot CH_2 \end{array}$$

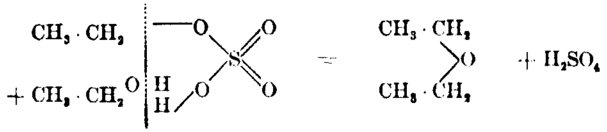
The ether of the laboratory is ethyl ether, often called sulphuric ether, although there is no sulphuric acid in its composition. Sulphuric acid, however, is employed in its manufacture from alcohol.

The reaction occurs in two stages:



Alcohol + Sulphuric acid. Ethyl sulphuric acid.

2. The ethyl sulphuric acid reacts with a second alcohol molecule.



The H_2O is not liberated but immediately goes to reconstruct the sulphuric acid.

This is an example of catalysis where the active agent in a reaction is not itself affected except perhaps temporarily as in this instance (p. 10).

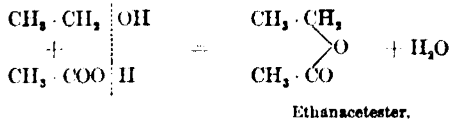
Theoretically therefore a given amount of H_2SO_4 should be able to convert an unlimited amount of alcohol into ether. But owing to secondary reactions the H_2SO_4 is in practice gradually exhausted.

The esters are the result of a reaction between the OH group of an alcohol and the OH group of an acid with loss of water, *i. e.*, of an organic base with an acid.

Example.

Ethanol.

Ethan acid.



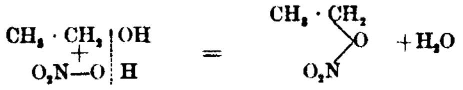
Ethanacetester.

As with the ethers the alcohols and acids are not necessarily corresponding homologues, so that one can have methylacetester, ethylpropanacidester, etc.

The acid may be mineral as well as organic.

Ethanol.

Nitric acid.



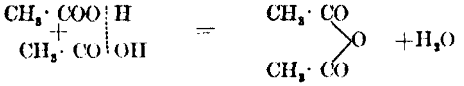
Ethyl nitrate.

The Anhydrids are formed by reaction between the OH groups of two acids with loss of water:

Example.

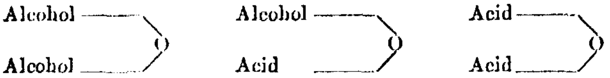
Acetic acid.

Acetic acid.



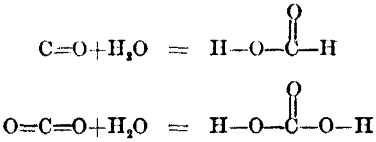
Acetanhydrid.

The ethers, esters and anhydrids may therefore be shown in diagrammatic form as follows:



Ether. Ester. Anhydrid.

The gases carbon monoxide $C = O$ and carbon dioxide $O=C=O$ may be regarded as anhydrids, since they may be obtained by driving off water from formic and carbonic acids.



Formic acid.

Carbonic acid.

It has been said that the C atom cannot support more than one OH group. This is true as a general rule but an exception must be made in the case of carbonic acid. Carbonic acid however only exists as such in solution in water, and is easily decomposed, CO_2 gas being driven off. It is therefore unstable having a tendency to throw off H_2O on the slightest provocation. Its salts however are stable, *e. g.*, K_2CO_3; $CaCO_3$.

It has also been said that every C atom must have all four valences occupied. Carbon monoxide is the single exception to this. It is formed on combustion with insufficient supply of oxygen.

Diatomic Alcohols.

A second group of a chain may become oxidized, so that two OH groups are present instead of one.

CH_3	CH_2OH	CH_2OH
CH_3	CH_3	CH_2OH
Ethane.	Ethanol (alcohol), monoatomic.	Ethandiol (glycol), diatomic.

Of these two OH groups one may become further oxidized to an acid, forming an oxyacid, and again both groups may become oxidized to form a diacid. The intermediate aldehydes need not be considered in detail.

CH_2OH	$COOH$	$COOH$
CH_2OH	CH_2OH	$COOH$
Ethandiol (glycol).	Ethanoxyacid, (oxyacetic acid), monobasic acid.	Ethandiacid, (oxalic acid), dibasic acid.

In the same way we may have propandiol and diacid, or butandiol and diacid, etc., propanoxyacid and so on. Graphic formulas of these and many others should be constructed in order to fully grasp the situation.

Triatomic Alcohols.

There may be three groups oxidized to OH and in this case we have a triatomic alcohol.

CH_2OH
$CHOH$
CH_2OH

Propantriol (glycerin or glycerol).

In like manner alcohols may be tetratomic, pentatomic, etc. Mannite is an alcohol with six C atoms all of which are oxidized to OH. It is therefore a hexatomic alcohol.

It is obvious that in the case of a diacid the acid radicals must be at either end of the chain since an acid radicle requires three valences =O and —OH, whilst two only are available in the middle C atoms, but with the oxyacids the alcohol OH may be attached to any of the C atoms. According to the position of the alcohol OH group with regard to the acid group the oxyacid is called alpha, beta, gamma, etc.

COOH	COOH	COOH	COOH
CH_2	α CHOH	α CH_2	α CH_2
CH_2	β CH_2	β CHOH	β CH_2
CH_3	γ CH_3	γ CH_3	γ CH_2OH
Butanacid (butyric acid).	α oxybutanacid (α oxybutyric acid).	β oxybutan (butyric) acid.	γ oxybutan (butyric) acid.

Lactic Acid is oxypropanacid and it is evident that the OH group may hold either one of two positions.

COOH	COOH
H C OH	H CH
CH_3	CH_2OH
α oxypropanacid (ethylidene lactic acid).	β oxypropanacid (ethylene lactic acid).

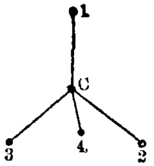
Asymmetric Carbon Atom and its Action on Polarized Light.

Taking the middle C of the α oxypropanacid we find that there are four different groups of atoms attached to it, COOH, CH_3, H and OH, whilst the middle C of the β acid has only three different kinds attached, COOH, CH_2OH and H.

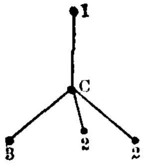
The former is an asymmetric C atom and the latter a symmetric C atom. Any C atom to which 2, 3, or 4 similar atoms or groups of atoms are attached is symmetric. Any C atom which has four *different* groups attached to it is asymmetrical.

If the molecule is regarded as existing in three planes

(stereo-chemistry), as no doubt is the case in nature, we can construct a diagram of it as follows :

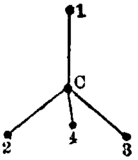


1. Asymmetric.

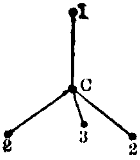


3. Symmetric.

If the attached groups are arranged in a different order as

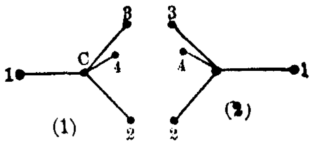


2. Asymmetric.



4. Symmetric.

it will be found that molecule No. 1 cannot be placed directly over molecule No. 2 in such a way that each group lies over a corresponding group. But if the molecules are turned over in opposite directions, the groups will then correspond. One is the mirror image of the other.



With a symmetric C atom (3) and (4), no matter how the two or more similar groups may be placed, one molecule can *always be superimposed upon another in such a way that each* group has a corresponding one underneath it. There is no necessity to turn the molecules over.

With an asymmetric C atom no matter how the groups are arranged one molecule can always be made to fit on another, provided they are turned over as shown in the diagram.

If a small model is made with wire and differently colored balls this fact is made obvious at a glance.

With an asymmetric C atom then there are always two different combinations possible, but no more.

One of these combinations when in solution always has the power of turning the plane of polarized light to the right (dextrorotatory) and the other to the left (lævorotatory). Why this is so is not clearly understood; the fact has to be accepted.

When an equal number of such right- and left-handed molecules are in solution together, they neutralize each other and the solution has no power over polarized light. It is the *inactive* or *racemic* form of the compound.

Alpha lactic acid may exist in three different forms, all of which give similar reactions, except that one is dextrorotatory (*d*) another lævorotatory (*l*) and the third inactive (*i*), whilst β lactic acid exists in one form only.

The α and β lactic acid are *chemical* isomers. The differences in their make-up are due to different chemical combinations. The differences between *d* and *l* lactic acid are simply differences of configuration. They are *physical* or *space* isomers.

Following up the question of the asymmetric carbon atom we may consider α, β dioxybutyric acid

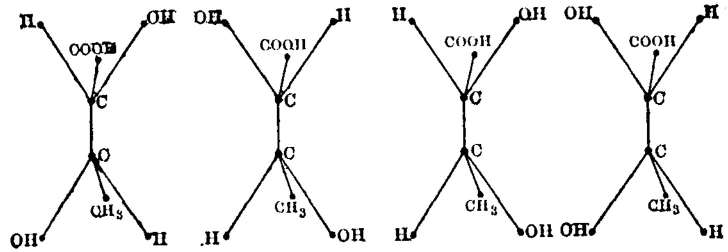
$$COOH.CHOH.CHOH.CH_3$$

and constructing its graphic stereochemical formula in three planes, find that it has two asymmetric C atoms.

No single one of these can be so placed over another that all the groups correspond, but if 1 and 2 are turned so as to face

each other their groups correspond. 1 is the mirror image of 2, and the same is the case with 3 and 4.

There are therefore two right-handed, two left-handed and two racemic forms, or we may say there are four space isomers.

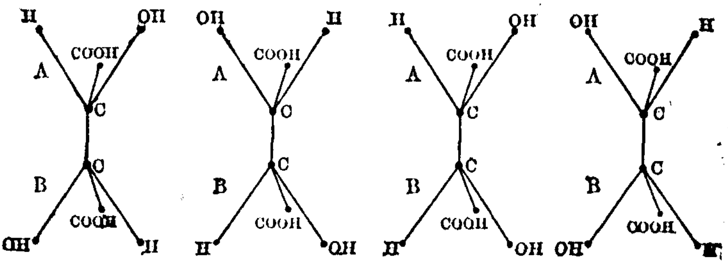


1 2 3 4

Tartaric acid is butandioldiacid,

COOH . CHOH . CHOH . COOH.

So this also has two asymmetric C atoms, but in this case each of the two central C atoms has similar groups attached, COOH, OH and H.



1 2 3 4

Taking 1 and 2 we find that the groups *A* attached to the upper C atom do not correspond with the groups *B* attached to the lower C atom. Nor do the groups *A* form a mirror image of the groups *B*. But the entire molecule 1 forms a mirror

image with the entire molecule 2. 1 and 2 are dextro (*d*) and lævorotatory (*l*) respectively, and together are inactive or racemic.

On studying 3 however we find that the groups *A* are the mirror image of the groups *B*. These two therefore neutralize each other by what is called *internal compensation* and the molecule in solution is inactive. The same is the case with 4, and since each of these is of itself inactive a mixture of the two is also inactive. 3 and 4, either singly or mixed, form only one single inactive compound, *mesotartaric acid.*

Tartaric acid therefore has three space isomers or four forms instead of six as with *β* dioxybutyric acid. The latter is said to be an *asymmetric molecule* whilst tartaric acid is a *symmetric* molecule.

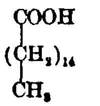
The dextrorotatory may be separated from the lævorotatory form in a racemic solution of tartaric acid :

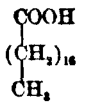
1. By sorting the crystals which are formed with some optically inactive substance. The *d* crystal has a slightly different form from the *l* crystal; one being a mirror image of the other.

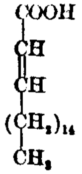
2. By the different solubilities of certain salts of the two forms. The strychnine salt is commonly used.

3. Certain moulds, *e. g.*, penicillium glaucum, if grown on a racemic solution, feeds on the *d* form and destroys it, leaving the *l* form untouched.

Fats.—Three of the higher monobasic (fatty) acids are of special interest in this connection and may be referred to here: They are:


Palmitic acid, $C_{16}H_{32}O_2$.


Stearic acid, $C_{18}H_{36}O_2$.


Oleic acid, $C_{18}H_{34}O_2$.

Oleic acid differs slightly from the other two in that it contains two unsaturated carbon atoms $C = C$.

The esters of these acids with the triatomic alcohol glycerin are the ordinary fats of the body. The combination occurs as follows:

$$\begin{array}{llll} H_2C-O-H & H-O-CO\cdot(CH_2)_{16}\cdot CH_3 & & H_2C-O-CO\cdot(CH_2)_{16}\cdot CH_3 \\ HC-O-H & +\,H-O-CO\cdot(CH_2)_{16}\cdot CH_3 & = & HC-O\cdot CO\cdot(CH_2)_{16}\cdot CH_3 + 3H_2O \\ H_2C-O-H & H-O-CO\cdot(CH_2)_{16}\cdot CH_3 & & H_2C-O-CO\cdot(CH_2)_{16}\cdot CH_3 \end{array}$$

Glycerin + 3 stearic acid, = Tristearin + 3 water.

$C_3H_5(OH)_3 + 3HSt. = C_3H_5St_3 + 3H_2O.$

Tripalmitin and triolein are formed in the same way. The fats of the animal body are composed of these three substances mixed in varying proportions. The greater the proportion of olein the lower the melting point of the fat.

Unsaturated compounds have a lower melting point than their corresponding saturated compounds. Olein therefore is a fluid at ordinary temperature whilst stearin and palmitin are solids.

Soaps. — Free fatty acid treated with an alkali or alkaline base will combine with it to form a *soap*.

Example.

$$\begin{array}{c} COOH \\ | \\ (CH_2)_{16} \\ | \\ CH_3 \end{array} + KOH = \begin{array}{c} COOK \\ | \\ (CH_2)_{16} \\ | \\ CH_3 \end{array} + H_2O$$

Potassium stearate (a soap).

Soaps are compounds formed by replacing the hydrogen of a fatty acid by a metal. Soaps of the alkali metals are soluble in water. Others, calcium soap, lead soap, etc., are insoluble.

On boiling fat with an alkali the latter will drive off the glycerin and form a soap (*saponification*)

$$C_3H_5St_3 + 3KOH = 3KSt + C_3H_5(OH)_3.$$

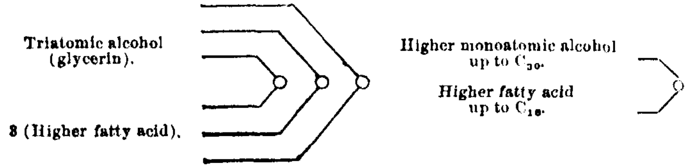
Stearin + Pot. hydroxide = Pot. stearate + Glycerin.

It is worth while to distinguish between the fats and the waxes.

The fats are the esters of glycerin and a higher monobasic acid.

The waxes are the esters of a higher monobasic alcohol and a higher monobasic acid.

Or diagrammatically,



Fats. Waxes.

The fats and oils are chemically alike. A fat when melted is called an oil, and a solidified oil a fat.

The true oils have of course nothing in common with the mineral oils which are *hydrocarbons and should, properly speaking*, be called paraffins.

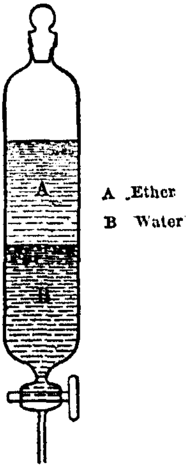


FIG. 4.

TESTS FOR FATS.

A. *Staining reactions.*

1. *Sudan* III. is a red stain which colors all fats but no other substances. It is insoluble in water, so has to be used in alcoholic solution.

2. *Osmic acid* stains olein black, but does not affect other fats. Since animal fats always contain more or less olein, osmic acid is useful for their demonstration, although for general purposes is not so reliable as Sudan III. It is generally used in 1 per cent. aqueous solution.

B. *Ether.* — The fats and free fatty acids (not the soaps) are soluble in ether, so that this is much used as a means of extracting fats. The tissue or whatever else it may be, is shaken well with ether in a test-tube — there are many forms of apparatus for doing this on a large scale and more thoroughly — and the ether then separated from the other material in a separating funnel (*Fig. 4*); the ether containing the fat in solution rises to the top.

The ether is collected in a porcelain dish, allowed to evaporate and the residue tested for fats, by staining and other reactions which need not be mentioned here.

Carbohydrates.

Carbohydrates.

Monosaccharides (simple sugars).	Disaccharides (compound sugars).	Polysaccharides (starches).

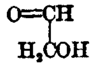
The carbohydrates are *normal* chains of C atoms containing H and O in the proportion of water.

Empirical formula of a carbohydrate $C_nH_{2x}O_x$.

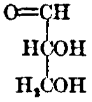
1. *Monosaccharides.* — The sugars are aldehydes or ketones with the remaining C atoms in the chain oxidized to alcohol (OH).

For convenience the sugars are all called by names ending in *ose* with a prefix indicating the number of C atoms.

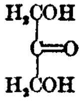
The simplest possible sugar is a biose.



Biose.



Triose (aldose).

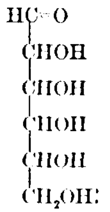


Triose (ketose).

and so on, tetrose, pentose, hexose, etc.

The only monosaccharides which occur in nature are certain pentoses and hexoses, of which the following are the best known.

Xylose (pentose-aldose).



Glucose (hexose-aldose).

Fructose (hexose-ketose).

The monosaccharides occurring in nature have all been synthesized in the laboratory and in addition to these a number of other pentoses and hexoses, besides trioses, tetroses, heptoses, octoses and nonoses.

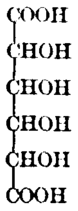
About 12 natural sugars are known and over 50 have been synthesized.

A glance at the graphic formula of a sugar will show that there are a large number of asymmetrical C atoms, and the number of possible combinations amounts to 16 with the hexaldoses and 8 with the hexketoses.

There are therefore 24 possible optically active hexoses besides the racemic forms. This can be easily verified by constructing graphic formulas, and it is worth while to do it.

The aldoses can be oxidized to acids. From glucose for instance three acids can be obtained.

Glyconic acid.



Saccharic acid.

Glycuronic acid.

Glyconic and glycuronic acid being, like the aldoses, asymmetric molecules with four asymmetric C atoms, each have 16 optical modifications. But saccharic acid is a symmetric molecule, so some of its forms are meso.

The meso forms are:

COOH	COOH	COOH
HCOH	HCOH	HCOH
HCOH	HCOH	HOCH
HCOH	HOCH	HCOH
HCOH	HOCH	HOCH
COOH	COOH	COOH

and the three forms which are the reverse of these, so there are three meso forms and ten optically active forms, 13 space isomers.

If glucose is oxidized in the laboratory the aldehyde group is first acted upon and glyconic acid is obtained. Further oxidation affects the alcohol group at the other end and saccharic acid is produced. The chain cannot be oxidized beyond this stage without breaking up.

In the body the aldehyde group appears to be protected in some way, so that the alcohol group at the other end is first oxidized and glycuronic acid is formed. On further oxidation in the body glycuronic acid breaks up. Glycuronic acid appears in the urine under conditions which will be explained later on.

The aldoses by virtue of their aldehyde group will reduce copper oxide (Fehling) as already described for aldehydes. It was said that this is an aldehyde and not a ketone reaction but a ketone containing a number of alcohol groups will react, so that the ketoses will reduce copper oxide or bismuth oxide as well as the aldoses.

Other sugar reactions will be discussed in Chapter V.

2. *Disaccharides.* — A disaccharide is a combination of two sugars with loss of H_2O. The empirical formula of a monosaccharide being $C_6H_{12}O_6$, that of a disaccharide is $C_{12}H_{22}O_{11}$.

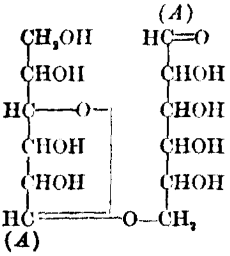
The more important disaccharides are:

Saccharose which is glucose + fructose,
Maltose which is glucose + glucose,
Lactose which is glucose + galactose.

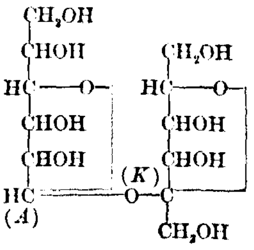
Maltose and lactose will reduce Fehling's solution but saccharose (cane sugar) will not.

The reason for this is that the latter is so constructed that the aldehyde group of the glucose and the ketone group of the fructose both enter into the combination, whilst with the two former one aldehyde group is left free in each case.

To illustrate this the construction of saccharose and maltose is given.



Maltose, one free aldehyde group.



Saccharose, no free aldehyde or ketone group.

The aldehyde and ketone groups are marked *A* and *K*.

3. *Polysaccharides.* — A number of monosaccharide or disaccharide molecules may be combined together to form polysaccharides and each time a fresh monosaccharide or disaccharide is added there is loss of H_2O so that the empirical formula of a polysaccharide is $(C_6H_{10}O_5)_n$. The polysaccharides are very

complex bodies and the number of sugar molecules which they contain is not known.

The principal polysaccharides are :

1. Cellulose. Fibrous and woody parts of plants.
2. Starch. Reserve material in seeds, roots and other parts of plants.
3. Glycogen or animal starch.
4. Dextrins. Bodies intermediate between any of these three and sugars.

Reactions of polysaccharides with iodine (Lugol's solution).

Starch gives a blue color.

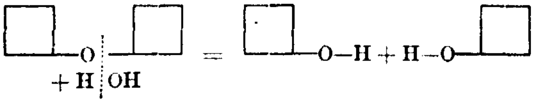
Glycogen gives a reddish brown color.

Dextrins (*a*) a red color (erythrodextrin).

(*b*) no color (leucodextrin).

The di- and poly-saccharides on boiling with dilute mineral acids, H_2SO_4 is generally used, are decomposed into monosaccharides by a process of hydrolysis. The acid acts as a catalyzer.

Hydrolysis is the breaking down of complex into simpler molecules with addition of water.



It must have been already noticed that H_2O plays a conspicuous part in reactions.

Two molecules may condense into one with loss of H_2O — "Dehydration." One molecule may split into two with addition of H_2O — "Hydration" or "Hydrolysis."

On building up the starch molecule from a monosaccharide there is dehydration, and on breaking it down into its component monosaccharides there is hydrolysis.

Cane sugar on boiling with acids is hydrolyzed to glucose and fructose.

The more complex insoluble starch molecule is hydrolyzed by a succession of steps through the dextrins and the disaccharide maltose, to glucose. The process is precisely similar to that which is carried out by the enzymes or ferments, amylase, and maltase, and is discussed more fully in Chapter VII.

Hydrolysis and Saponification.

We may digress a little here to point out the differences between hydrolysis and saponification. The terms are only used for reactions with organic compounds.

Hydrolysis is simplification with absorption of water.

Saponification is simplification with absorption of a hydroxide.

Hydrolysis may take place at the point of junction between any two molecules forming a complex, whether alike or not, whether acid, basic or neutral, since the agent of hydrolysis, H_2O, is neutral.

Saponification can only take place when one of the molecules forming the complex is an organic acid, since the agent of saponification KOH or other hydroxide, is a base and can only react with an acid.

Examples of hydrolysis.	Examples of saponification.
1. Glycerin radical $\langle$ O—St., O—St., O—St. $+ 3H_2O$	Glycerin radical $\langle$ O—St., O—St., O—St. $+$ KOH
Fat = glycerin + 3 (stearic acid).	= glycerin + 3 (potassium stearate, a soap).
2. Glucose radical—O—glucose radical $+ H_2O$	glucose radical—O—glucose radical $+$ KOH
Maltose = glucose + glucose.	= no reaction.
3. $CH_3 \cdot CO$ / $CH_3 \cdot CH_2$ $\rangle$ O $+ H_2O$	$CH_3 \cdot CO$ / $CH_3 \cdot CH_2$ $\rangle$ O $+$ KOH
(Ethylacetester) = $CH_3 \cdot COOH + CH_3 \cdot CH_2OH$, (acetic acid + alcohol).	= $CH_3 \cdot COOK + CH_3 \cdot CH_2OH$, (potassium acetate + alcohol).
4. $CH_3 \cdot CH_2$ / $CH_3 \cdot CH_2$ $\rangle$ O $+ H_2O$	$CH_3 \cdot CH_2$ / $CH_3 \cdot CH_2$ $\rangle$ O $+$ KOH
Ethylether = $CH_3 \cdot CH_2OH + CH_3 \cdot CH_2OH$.	= no reaction.

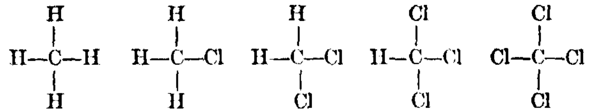
Other hydroxides, NaOH, $Ca(OH)_2$, $Ba(OH)_2$, etc., saponify equally well. We may have calcium soaps, lead soaps and so on. Any esters can be saponified (example 3) although the resulting compound may not be what we commonly regard as a soap.

The terms however are somewhat loosely used. The organic chemist seldom makes any distinction between hydrolysis and saponification, the latter term being often used to describe a process which is in reality hydrolysis. For instance the "saponification" of fats by superheated steam, or of an ester by the action of an acid. In each case an acid, not a salt, is one of the products, so that strictly speaking this is hydrolysis.

CHAPTER IV.

A. Halogen Derivatives of Carbon Compounds.

If CH_4 is treated with chlorine gas it displaces one or more of the H atoms, according to the amount of chlorine used.



Methane. Monochlormethane. Dichlormethane. Trichlormethane (chloroform). Tetrachlormethane.

Similar compounds are formed with bromine and iodine.

Chloroform is trichlormethane $CHCl_3$,
Iodoform is triodomethane CHI_3,
Bromoform is tribromethane $CHBr_3$.

Iodoform can be made from alcohol on addition to the latter of a solution of iodine in NaOH and heating.

$$C_2H_5OH + 6NaOH + 4I_2 = CHI_3 + HCOONa + 5NaI + 5H_2O$$

This reaction is used as a test for alcohol in solution, the merest trace of iodoform being perceptible by its odor. If alcohol is present in appreciable quantities iodoform separates out as a yellow precipitate.

B. Sulphur Compounds.

S and O are interchangeable in a variety of compounds. The interchange may take place in the alcohol (OH) groups of oxidized carbon compounds with formation of mercaptans.

$$\underset{\text{Ethanol.}}{\begin{array}{c}H_2COH\\|\\CH_3\end{array}} + H_2S = \underset{\text{Thioethanol (ethylmercaptan).}}{\begin{array}{c}H_2CSH\\|\\CH_3\end{array}} + H_2O$$

Methyl, propyl, etc., mercaptans can be formed in the same way. The mercaptans are recognizable by their garlic odor, and the above reaction may be used as a test for alcohols. Direct H_2S however cannot be used. The reaction is obtained indirectly as follows:

$$5C_2H_5OH + P_2S_5 = 5C_2H_5SH + P_2O_5.$$

C. Nitrogen Compounds.

The protoplasm of the living body yields fats, carbohydrates and proteids. The fat and carbohydrate molecules, already discussed, contain no N, but all proteids, their modifications and derivatives contain it, so that a consideration of the combinations of nitrogen and carbon is in fact an introduction to the study of proteids, and therefore of special importance to the physiological chemist.

Nitrogen (N) has three or five valences. In the latter case one of the attached groups is usually OH, or represents OH.

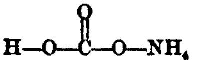
$N \equiv H_3$	$H - O - N \equiv H_4$	$Cl - N \equiv H_4.$
Ammonia.	Ammonium hydroxide.	Ammonium chloride.

Introductory.

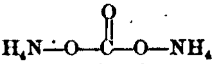
Carbon dioxide gas, CO_2, in solution takes up H_2O to form carbonic acid, $CO(OH)_2$.

Ammonia, NH_3, in solution takes up H_2O to form ammonium hydroxide, NH_4OH.

Carbonic acid and ammonium hydroxide will combine together to form ammonium carbonate and water.



Monoammonic carbonate.



Diammonic carbonate.

The OH of monoammonic carbonate may be replaced by other groups, a chain of C atoms for instance, and the formula may be written as

$$R-\overset{\displaystyle O}{\overset{\|}{C}}-O-NH_4$$

R being any organic radical. Suppose R is CH_3, we then have $CH_3 - COONH_4$ or ammonium acetate.

This may also be called methylammonium carbonate.

If $R - COONH_4$ is deprived of H_2O we have

$$R-\overset{\displaystyle O}{\overset{\|}{C}}-NH_2$$

an amid.

If

$$R-\overset{\displaystyle O}{\overset{\|}{C}}-NH_2$$

is deprived of H_2O we have $R-C\equiv N$ a nitril or cyan compound.

The difference between nitrils, amids, and ammonium carbonate is merely a matter of less or more water in combination, so that they are evidently closely related.

Nitrils.

Hydrocyanic acid	$H - C \equiv N$,
Isohydrocyanic acid	$H - N \equiv C$.

On oxidation :

Cyanic acid	$H - O - C \equiv N$,
Isocyanic acid	$H - N = C = O$,

Examples of salts.

Potassium cyanide	$K - C \equiv N$,
Potassium cyanate	$K - O - C \equiv N$.

The highly poisonous nitrils or cyan compounds readily take up sulphur, forming with it harmless so-called rhodanid compounds, or more commonly sulphocyanides.

$$HCN + S = H - S - CN.$$

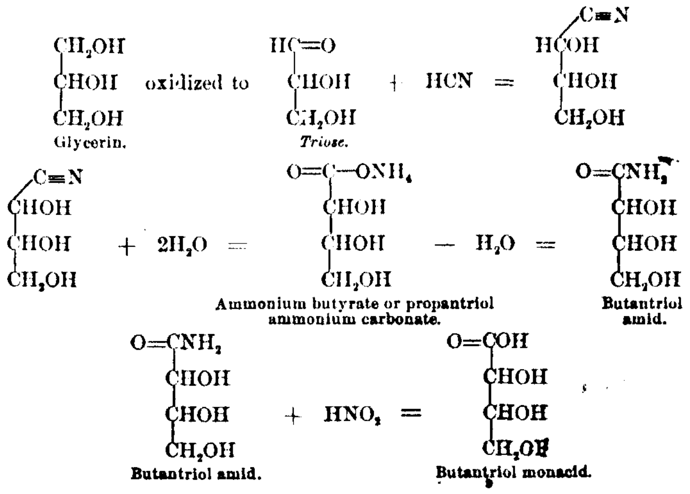
Thiocyanic acid.

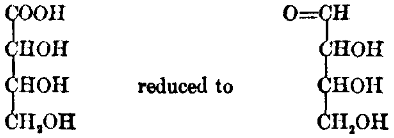
Thiocyanic acid itself is somewhat poisonous, but its salts are not.

Isothiocyanic acid $H - N = C = S$ bears the same relation to isocyanic acid as thiocyanic acid does to cyanic acid. In each case S takes the place of O (cf. mercaptans).

The isocyan compounds, isomers of the cyan acids, are also called acids but they are not true acids. They do not form stable salts with bases, but enter into combination with certain other substances in a characteristic manner. They will be referred to again.

$R - C \equiv N$ readily attaches itself to an aldehyde group and by this means sugars have been synthesized. Starting with glycerin the following graphic formulas explain the process:





Butantriol monacid. Butantriol monal *Tetrose.*

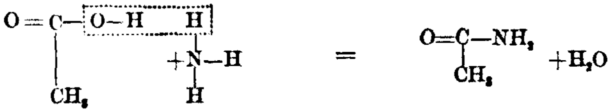
A triose has been converted into a tetrose, and by the same process a tetrose can be converted into a pentose, a pentose into a hexose. Sugars have been synthesized in this way up to a nonose.

It has to be taken for granted that chemists can reduce, oxidize, hydrate, dehydrate, etc., at will within certain limits. To enter into details of the methods employed would be out of place here.

AMIDS, AMINS, AMINO ACIDS.

1. *Amids.* — An amid is the result of a reaction between the OH group of an *acid* and NH_3.

Example :



Acetic acid, $CH_3 \cdot COOH$ Acetamid, $CH_3 \cdot CONH_2$

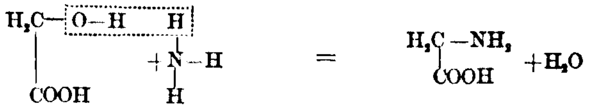
2. *Amins.* — An amin is the result of a reaction between the OH group of an *alcohol* and NH_3.

Example :

Ethylalcohol, $CH_3 \cdot CH_2OH$ Ethylamin, $CH_3 \cdot CH_2NH_2$

3. *Amino (amido) acids.* — An amino acid is the result of a reaction between the alcohol (OH) group of an *oxyacid* and NH_3.

Example:



Oxyacetic acid, $CH_2OH \cdot COOH$. Aminoacetic acid, $CH_2NH_2 \cdot COOH$.

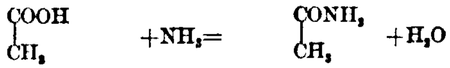
These reactions are intended merely to show the structure of the compounds and do not represent the methods by which they are formed in the laboratory.

The prefix *am* always denotes an $-NH_2$ group. For an $=NH$ group the prefix *im* is used. Thus we may have imids and imins. How these arise will become clear as we proceed.

Amids.

An amid may be formed from any of the organic acids.

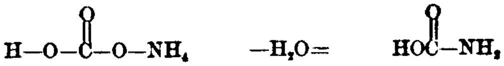
Example:



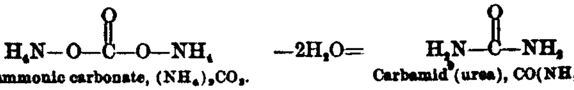
Ethan acid (acetic acid). Ethan amid (acetic amid).

and similarly for the higher homologues, propanamid, butanamid, etc.

But the most important for us is the diamid urea. Both it and the monamid carbamic acid may be formed from the ammonium carbonates with loss of H_2O.



Monoammonic carbonate, $H(NH_4)CO_3$. Carbamic acid, HCO_2NH_2.



Diammonic carbonate, $(NH_4)_2CO_3$. Carbamid (urea), $CO(NH_2)_2$.

Urea was the first of the organic compounds synthesized. Wöhler accomplished it in 1828 by the following reaction.

$$H{-}N{=}C{=}O + NH_4OH = NH_4{-}N{=}C{=}O + H_2O$$

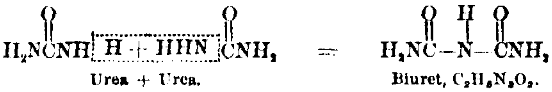
Isocyanic acid + Ammonium hydroxide = Ammonium isocyanate.

But as already mentioned isocyanic acid does not form stable salts, so ammonium isocyanate falls to pieces and reconstructs itself as its more stable isomer urea.

It will be seen that the empirical formula of each is CH_4N_2O.

BIURET.

1. On melting crystals of urea by heating, a part of the molecules condense with loss of NH_3 forming the soluble *biuret.*

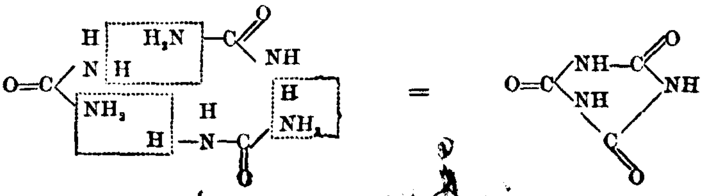


Urea + Urea. Biuret, $C_2H_5N_3O_2$.

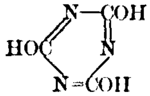
On treating a solution of this with NaOH and a very dilute solution of $CuSO_4$ it gives the well-known biuret reaction, a pink to violet color.

Proteid material gives the biuret reaction, although proteid contains no biuret. It contains however a radical which is so closely allied to biuret that it affords the same reaction, but this will be discussed more in detail later on.

2. Another part of the urea molecules condense still further to an insoluble compound, cyanuric acid.

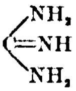


The latter compound is unstable and rearranges itself as



Cyanuric acid.

Guanidin is



and this can be changed to urea by boiling with baryta water.

$$HNC(NH_2)_2 + H_2O = CO(NH_2)_2 + NH_3$$

Guanidin. Urea.

The NH is driven out and replaced by O.

Since guanidin exists in combination in the proteid molecule, a part of the urea excreted is no doubt formed by some such reaction as this. But the amount of guanidin in proteid is insignificant and only accounts for a very small part of the urea actually excreted. The theories of urea production will be discussed later on.

Amid N.

In reading articles on physiological chemistry one often meets with the expression *Amid N* or *Ammonia N.* The NH_2 group of an amid can be split off as NH_3 on boiling with NaOH or some other alkaline base. The stronger base drives out the weaker base NH_3 from the acid radical.

$$CO(NH_2)_2 + 2NaOH = Na_2CO_3 + 2NH_3.$$

Urea. Sodium carbonate.

The NH_2 groups of the amins and amido acids replace an alcohol OH group with which an alkaline base has no affinity, so they are not driven off in this way and the nitrogen so com-

bined is called *stable N.* It cannot be obtained directly as NH_3. The whole molecule must be broken down by drastic methods, by the use, for example, of con. H_2SO_4 and $KHSO_4$, and the N obtained as an ammonium salt. This is the basis of the Kjeldahl method of determining N content.

The group NH_3 although basic seems to have affinities for OH groups apart from its basic qualities. When it exists in solution as NH_4OH it acts as a base only. Thus we can have

$$\begin{array}{l} H_2CNH_2 \\ \;\;| \\ \;\;CH_3 \end{array}$$

but not

$$\begin{array}{l} H_2C—O—NH_4 \\ \;\;| \\ \;\;CH_3 \end{array}$$

THE AMINS.

As a result of the action between the OH of an alcohol and NH_3 the O is lost.

$$R - COH + NH_3 = R - CNH_2 + H_2O.$$

So that the amins appear as paraffin chains with NH_2 attached.

Two, three or more NH_2 groups may be attached to a chain and in this case the compounds are called diamins, triamins, etc.

H_2CNH_2 CH_3	H_2CNH_2 H_2CNH_2	H_2CNH_2 $HCNH_2$ $HCNH_2$ CH_3
Ethanmonamin (ethylamin).	Ethandiamin (ethylendiamin) or dimethylendiamin.	Butantriamin, (butylentriamin) or tetramethylentriamin.

Again a second H of NH_3 may combine with another chain, forming a secondary amin. If all three hydrogen atoms of the NH_3 are displaced the compound is a tertiary amin.

Primary (ethylamin).	Secondary (diethylamin).	Tertiary (triethylamin).
$CH_3 \cdot CH_2NH_2$	$\begin{matrix} CH_3 \cdot CH_2 \\ \quad\diagdown \\ \quad\; NH \\ \quad\diagup \\ CH_3 \cdot CH_2 \end{matrix}$	$\begin{matrix} CH_3 \cdot CH_2 \\ \quad\diagdown \\ CH_3 \cdot CH_2 - N \\ \quad\diagup \\ CH_3 \cdot CH_2 \end{matrix}$

Any of the alcohols may combine with NH_3 so that we may have for example, methyl or propylamin, etc., among the primary; methylethylamin, ethylpropylamin, etc., among the secondary; dimethylethylamin, trimethylamin, etc., among the tertiary amins.

Reactions of the Amins.

1. *Carbylamin* (Hofmeister's).

Treated with chloroform ($CHCl_3$) and alcoholic KOH, the primary (NH_2) amins undergo a peculiar change:

$$\underset{\text{Ethylamin.}}{\begin{matrix} H_2C\,NH_2 \\ | \\ CH_3 \end{matrix}} + CHCl_3 + 3KOH = 3KCl + 3H_2O + \underset{\text{Ethylcarbylamin.}}{\begin{matrix} H_2C - N \equiv C \\ | \\ CH_3 \end{matrix}}$$

The K combines with the Cl. This liberates three OH radicals (hydroxyls) which seize the remaining H from the C of the chloroform, and the H_2 from the N of the amin, to form $3H_2O$. The N and the C are thus left unsatisfied, so the C immediately combines with the N, which in a most accommodating manner increases its atomicity from 3 to 5, allowing the C to saturate itself with the four spare valences.

The carbylamins are recognizable by their nauseating odor which once smelt is never forgotten.

2. *Mustard Oil* (Hoffman's).

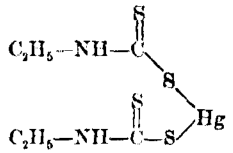
The primary amins treated with carbon disulphide, $S = C = S$ (cf. carbon dioxide, $O = C = O$) afford the following:

$$H_2\underset{\displaystyle CH_3}{C}NH_2 + CS_2 = CH_3\cdot CH_2NH-\overset{\displaystyle S}{\overset{\|}{C}}-S-H$$

Ethylamin.

The C of the CS_2 joins the N, and the displaced H joins the S which has been deprived of one valence.

This compound is now treated with mercury (Hg), say HgO, with the following result:



$+H_2O$

But this is a very unstable compound and at once breaks up of itself into

$$2(C_2H_5 - N = C = S) + HgS + H_2S.$$

Ethyl isothiocyanate (a mustard oil).

The mustard oils are the isothiocyanates (isothiocyanesters) of alcohols and are recognizable by their pungent odor.

The secondary and tertiary amins do not afford the above reactions because they do not contain enough hydrogen atoms to enable the changes to take place. This can be made evident on attempting to work it out by means of graphic formulas and equations.

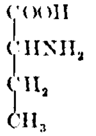
Amino Acids.

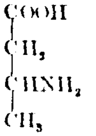
To the physiological chemist the amino acids are the most important of the nitrogen compounds since the proteid molecule is almost entirely composed of them.

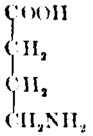
Since they are derived from the alcoholic OH group of the oxyacids we may recall the fact that such an OH group may be

attached to any of the C atoms in a chain, and according to its position as regards the COOH group is called alpha, beta, gamma, etc.

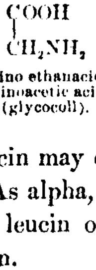
Just so with the amino acids:

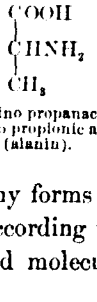

α amino butyric acid.

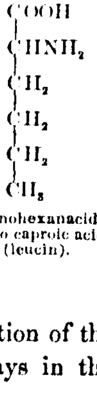

β amino butyric acid.


γ amino butyric acid.

The most important of the amino acids are:


Amino ethanacid, aminoacetic acid (glycocoll).

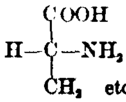

Amino propanacid, amino propionic acid (alanin).


Aminohexanacid, amino caproic acid (leucin).

Leucin may occur in many forms:

1. As alpha, beta, etc., according to the position of the NH_2. The leucin of the proteid molecule is always in the alpha position.

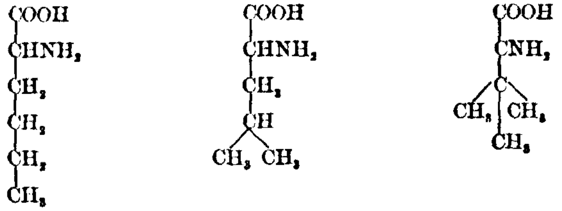
2. Since the C to which the NH_2 is attached is asymmetric.


etc.

leucin may be dextro- or lævorotatory.

The leucin of the proteid molecule is nearly always lævorotatory.

3. The chain may be normal, iso or meso.



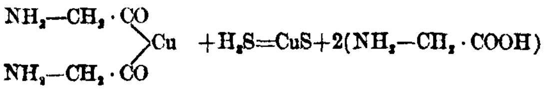
Normal. Iso. Meso.

The leucin of the proteid molecule is nearly always iso, and is called isobutylaminoacetic acid. But it is perhaps easier to think of it as alpha amino caproic acid.

Reactions of the Amino Acids.

The amino acids all give insoluble azure blue salts with copper, and in this way can be isolated as a group in crystal form.

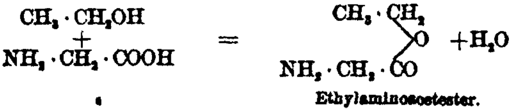
The crystals are then dissolved in hot H_2O and H_2S passed through :



The insoluble CuS falls as a precipitate, leaving the amino acid in solution.

Separation of Amino Acids from Each Other.

The amino acids very readily form ethylesters.



Ethylaminoacetester.

The ethylesters of the amino acids all have well-defined boiling points so can be separated by fractional distillation. But being very unstable the distillation must be carried on "in vacuo" at low temperatures or the esters will break up.

Certain characteristics of the amids, amins, and amino acids may be considered side by side for purposes of comparison.

1. *Reaction.*

The amids are neutral.

A base NH_3 combines with the OH of an acid (cf. mineral salts).

The amins are basic.

There is no acid O in the molecule to neutralize the base NH_2.

The amino acids are both acid and basic.

Since neither the acid nor the base are in direct combination each of them retains its own characteristics. The amino acids are acid by virtue of their COOH group and at the same time basic by virtue of their NH_2 group.

2. *Action of Nitrous Acid.*

Nitrous acid (HNO_2) acts in the same way on all NH_2 groups whether ~~amid, amin, or amino~~. It has the power of substituting OH for the NH_2 group, at the same time breaking up the latter and liberating free N.

The reaction occurs as follows :

1
$$\begin{array}{l} R-\overset{O}{\overset{\|}{C}}-\,\vdots\,N\,\vdots\,H_2 = R-\overset{O}{\overset{\|}{C}}-O-H+N_2+H_2O \\ \quad + HO\,\vdots\,N\,\vdots\,O \end{array}$$

Amid+nitrous acid, $R-CONH_2+HNO_2$. Acid, $R-COOH+N_2+H_2O$.

2
$$\begin{array}{l} R-CH_2-\,\vdots\,N\,\vdots\,H_2 = R-CH_2OH+N_2+H_2O \\ \quad + HO\,\vdots\,N\,\vdots\,O \end{array}$$

Amin. Alcohol.

3

Amino acid, R—CHNH$_2$·COOH. Oxyacid, R—CHOH·COOH.

HNO_2 is unstable, passing off quickly into NO and NO_2 gases (brown fumes) with loss of H_2O, so has to be used in a nascent condition.

To obtain this potassium nitrite, KNO_2, is added to the solution to be tested and then H_2SO_4:

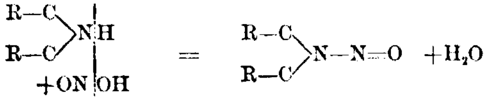
$$2KNO_2 + H_2SO_4 = K_2SO_4 + 2HNO_2.$$

The liberated HNO_2 immediately attacks the NH_2 groups. It is its desire to get rid of its OH groups which is the cause of this. If left to itself:

(N_2O_2 usually called NO.) (N_2O_4 usually called NO_2.)

and goes off in brown fumes, but it is able to get rid of its OH more quickly by attacking NH_2 groups.

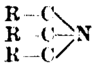
If, however, HNO_2 comes in contact with an NH (secondary or imid group) the action is different.



Secondary NH group. Nitroso combination.

The HNO_2 parts with its OH which combines with the single H to form H_2O, and the two N's combine with each other. In this case there is nothing left for the O, so it remains attached to the N and a so-called nitroso compound results. The nitroso $= N - N = O$ reaction is often of value for the detection of NH groups.

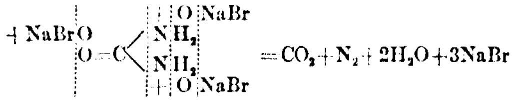
HNO_2 has no effect on a triamin.



having no H cannot assist HNO_2 to get rid of its OH, so the latter goes off in brown fumes in the ordinary way, leaving the triamin untouched.

Sodium hypobromite, NaOBr, in alkaline solution will also split off N from NH_2 groups, though not so completely as HNO_2. About 90 per cent. of the N may be obtained in this way.

The reaction with urea occurs as follows:



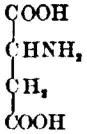
This test is simple and much used in clinical pathology for estimation of the amount of urea in urine. But it is not accurate enough for quantitative analysis and the scientific chemist leaves it severely alone.

The test is made in a fermentation tube and the amount of gas evolved measured as N, the CO_2 being absorbed by the alkali to form carbonates.

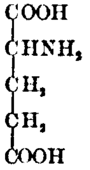
Other Amino Acids.

Besides the monoamino-monobasic acids the proteid molecule contains a small amount of:

1. *Monoamino-dibasic Acids.* — Of these the most important are:



Amino butandiacid, asparaginic acid.



Amino pentandiacid glutaminic acid.

These contain two COOH groups to one NH_2, so are distinctly acid. Asparagin is sodium asparaginate

$$COONa.CHNH_2.CH_2.COONa.$$

2. *Diamino Monobasic Acids.*

COOH
α $CHNH_2$
β CH_2
γ CH_2
δ CH_2NH_2

Diamino pentanacid
α, δ, diamino valerianic acid
(ornithin).

COOH
α $CHNH_2$
β CH_2
γ CH_2
δ CH_2
ε CH_2NH_2

Diamino hexanacid
α, ε diamino caproic acid
(lysin).

These contain two NH_2 to one COOH, so are distinctly basic. It will be observed that, like leucin, all the above are alpha amino acids. In addition ornithin contains NH_2 in the delta, and lysin in the epsilon position, so they are called *α*, *δ* amino valerianic acid and *α*, *ε* amino caproic acid respectively.

Glycosamin.

Sugar occurs in combination in the proteid molecule as glycosamin.

$$CHO.CHNH_2.CHOH.CHOH.CHOH.CH_2OH.$$

Glycosamins can occur as insoluble polymers (cf. glucose and glycogen) and in this form, called chitosamin, mixed with lime salts, constitute the hard chitinous covering of lobsters and other crustaceæ.

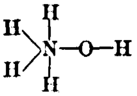
Chitosamin does not occur in vertebrate animals. Their bones are modified proteids (gelatin, collagen) mixed with lime salts.

Cholin. — Before leaving the subject of the N compounds the constitution of cholin may be given.

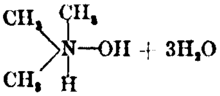
Cholin is trimethyl oxethyl ammonium hydroxide.

Knowing this it is easy to construct its graphic formula:

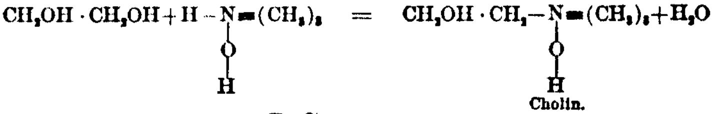
Ammonium hydroxide is



Three of the hydrogen atoms react with CH_3OH, making:



The remaining H reacts with ethandiol (oxethyl alcohol) (glycol).



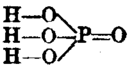
Cholin.

P Compounds.

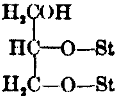
Phosphorus occurs in combination in the proteid molecule in the shape of lecithin.

Lecithin is diolein or dipalmitin or distearin cholin phosphoric acid and with the knowledge already gained its formula can be constructed.

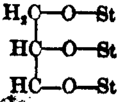
Taking the distearin form as an example:



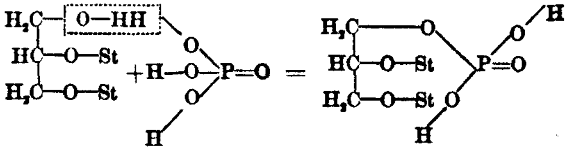
Phosphoric acid.



Distearin.

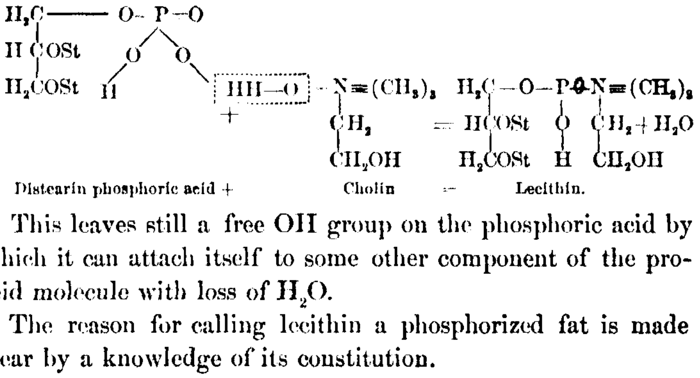


Tristearin.

1. 

Distearin + Phosphoric acid. Distearin phosphoric acid.

2.



Distearin phosphoric acid + Cholin = Lecithin.

This leaves still a free OH group on the phosphoric acid by which it can attach itself to some other component of the proteid molecule with loss of H_2O.

The reason for calling lecithin a phosphorized fat is made clear by a knowledge of its constitution.

CHAPTER V.

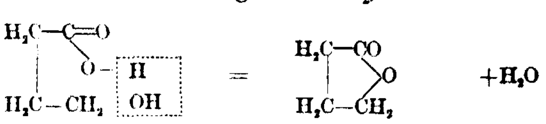
CYCLIC COMPOUNDS.

I. THE chains of C atoms have a tendency to curl over and join at the two ends, forming in this way a closed chain. In order that the chains may close up, there must be groups at either end which would naturally have a tendency to combine with each other.

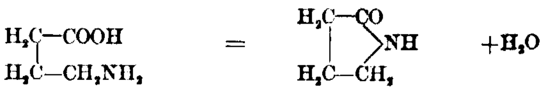
An acid COOH group would have a tendency to combine with an alcohol to form an ether, or with an NH_2 group to form an imid, NH.

A chain therefore with COOH at one end and NH_2 at the other would be likely to close up if the chains were bent over sufficiently so that the two would come together.

The shorter chains, amino acetic acid, amino propionic acid, are not able to bend over sufficiently to meet. But γ amino butyric acid is long enough, and the same may be said of all amino acids where the NH_2 group is above β. If an alcohol group joins with a COOH group the compound is called a Lactone, and COOH combining with NH_2, a Lactam.



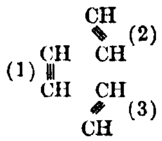
γ oxybutyric acid. γ oxybutyric lactone (an autoester).



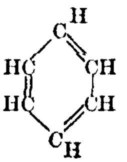
γ amino butyric acid. γ amino butyric lactam (an auto aminester).

II. Again two or more separate molecules of the same substance may condense with loss of H_2O or NH_3 and form a closed chain. Example, cyanuric acid (page 60).

III. Two or more similar unsaturated compounds may interchange a valence and thus form a closed chain. Ethine (acetylene gas) is such a compound $HC \equiv CH$. If three molecules of ethine are arranged as follows :



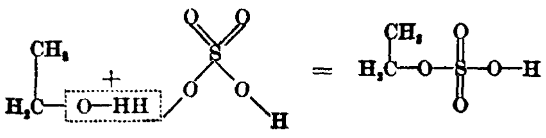
It can easily be seen that if (1) gives up a valence to (2), (2) to (3) and (3) to (1) the resulting molecule will appear as,

 or C_6H_6

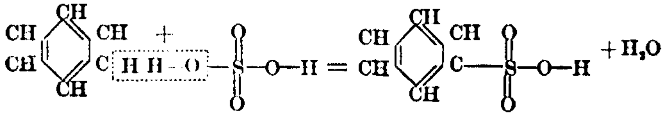
This, by far the most important of the cyclic compounds, is a benzene ring, and on the benzene ring, as a basis, an infinite variety of molecules can be built up.

Benzene may combine with an acid.

In the case of an alcohol combining with an acid to form an ester there is always an O between the two molecules.

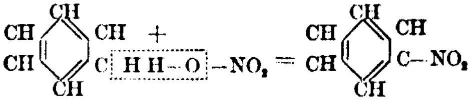


But if benzene combines with sulphuric acid the S joins directly on to the C without any intermediate O, and the product is called a sulphonic acid.



Benzene, C_6H_6. Benzol sulphonic acid, $C_6H_5 \cdot SO_3H$.

In the same way benzene treated with nitric acid, HNO_3

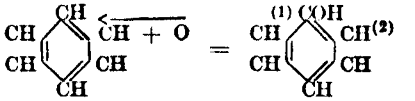


Benzene, C_6H_6. Nitrobenzene, $C_6H_5 \cdot NO_2$.

Nitrobenzene is the basis on which explosive substances are built up.

Oxybenzenes.—Any of the H atoms attached to the benzene ring can be oxidized to OH, but obviously no further, since there is only one valence available for oxidation. (Cf. Tertiary alcohols, p. 29.)

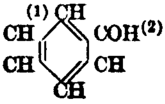
If one H is oxidized :



Benzene, C_6H_6. Oxybenzene (Carbolic acid) (Phenol), C_6H_5OH.

It makes no difference which of the H atoms is oxidized, the product is always the same.

Supposing the OH group to be on (2) instead of (1)



It is obvious that on turning the page on which this is printed one sixth of the way round (2) will be where (1) was before and the molecule appears as in the first example given.

Benzene cannot be directly oxidized to phenol in the laboratory, but can be produced artificially in a round-about way.

For convenience the CH groups and the double links are left out in constructing a benzene ring.

1.

Benzene, C_6H_6. Nitrobenzene, $C_6H_5 \cdot NO_2$.

On treating nitrobenzene with nascent H the NO_2 is reduced to NH_2

2.

Nitrobenzene, $C_6H_5 \cdot NO_2$. Phenylamin (Anilin), $C_6H_5 \cdot NH_2$.

Nascent H may be produced by stannous chloride and an alkali

$$\begin{array}{l} Sn-Cl \\ \quad | \\ Sn-Cl \end{array} + 2NaOH = 2SnO + 2NaCl + H_2$$

(For other methods of obtaining H see p. 14.)

The NH_2 group of phenylamin is affected by HNO_2 in precisely the same way as NH_2 groups of the open chain amins already discussed.

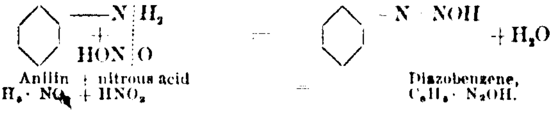
3.

Phenylamin + nitrous acid = Phenol.
$C_6H_5 \cdot NH_2 + HNO_2$ = $C_6H_5 \cdot OH$.

The final product being artificial phenol.

But if this last process is conducted at a very low temperature, below freezing point, the reaction is different; somewhat analogous to the nitroso reaction.

In this case the N does not leave the benzene ring but joins itself to the N of the HNO_2 with two valences.



Diazobenzene is the basis for anilin dyes and if our object were to study the anilin dyes we could follow up the subject in this direction. But the anilin dyes do not concern us here.

The process of obtaining phenol may be summarized:

1. C_6H_6	Benzene
2. $C_6H_5 . NO_2$	Nitrobenzene
3. $C_6H_5 . NH_2$	Anilin
4. $C_6H_5 . OH$	Phenol

The alcohol OH group of an open chain is basic (p. 36).

The alcohol group of a benzene ring is slightly acid. In consequence of this, their reactions are somewhat different.

Phenol for example will not form esters with an acid.

Millon's Reaction.

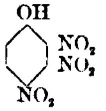
If phenol is heated with Millon's reagent (which is a mixture of mercuric and mercurous nitrate containing some free nitrous acid) a red color is produced.

The color is the result of a reaction between the reagent and a single free OH group attached to a benzene ring. No matter what else is attached to the ring, so long as there is a single free OH group the substance will afford the reaction.

Most proteid material gives Millon's reaction, indicating that in its composition there is a benzene ring with one free OH group attached.

It has been said that nitrobenzene is the basis of explosives. Since the study of explosives is not our object this branch will

not be followed up, but one nitro compound may be mentioned,



Trinitro oxybenzene (Picric acid).

The presence of so much O makes the OH group a strong acid in this case so that picric acid will combine with bases much more readily than phenol.

Picric acid has a bright yellow color and this is a characteristic of all nitrobenzenes.

Xanthoproteic Reaction.

Proteids give a yellow color on warming with nitric acid.

This indicates the presence of a benzene ring; a nitrobenzene being formed.

In this case it is not necessary, as with Millon's reaction, for the benzene ring to have an OH group attached.

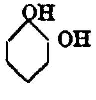
Dioxybenzenes.

A second OH group of a benzene ring may be oxidized and in this case we have a dioxybenzene.

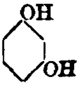
According to the position of the OH groups as regards each other there are three possible different combinations among the dioxybenzenes.

Each combination forms a distinct compound which differs in some of its reactions from the other two.

The three different dioxybenzenes are:



Orthodioxybenzene (Catechin), $C_6H_4\cdot(OH)_2$



Metadioxybenzene (Resorcin), $C_6H_4\cdot(OH)_2$

Paradioxybenzene (Hydrokinone), $C_6H_4\cdot(OH_2)$

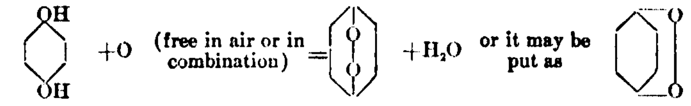
The empiric formula of each is the same, but the arrangement of the OH groups on the ring is different. If the two OH groups lie next to each other the compound is designated as "ortho." If one CH group lies between the two OH groups as it is called "meta." If two CH groups lie between the two OH groups it is called "para."

The dioxybenzenes are reducers, *i. e.*, they have the power of abstracting O from certain other compounds with the formation of quinones.

In the presence of alkalis they can take up O from the air and form quinone.

The metadioxybenzene has little power of doing this. It is a feeble reducer.

The ortho-dioxybenzene is stronger, but the para is the most active reducer of the three



Paradioxybenzene hydrokinone, $C_6H_4\cdot(OH)_2$. = Quinone, $C_6H_4O_2$.

Reactions.

All the dioxybenzenes give an emerald green color with $FeCl_3$. Adrenalin gives an emerald green color with $FeCl_3$ and this is due to the presence in it of catechin in combination.

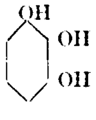
Catechin alone does not raise the blood pressure, but in combination with some other substance the exact constitution of which is not certain, forms the active principle of adrenalin.

The dioxybenzenes do not give Millon's reaction. This only occurs with substances which have a single OH group on the ring.

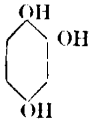
Resorcin is used as a test for ketoses. Its crystals are added to a solution containing a ketose and warmed. A red color and later brown precipitate soluble in alcohol is formed.

TRIOXYBENZENES.

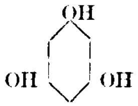
Three of the CH groups of benzene may be oxidized and, as with the dioxybenzenes, different compounds are formed according to the positions of the OH groups.



Ortho trioxybenzene.
Pyrogallol.



Meta
trioxybenzene.

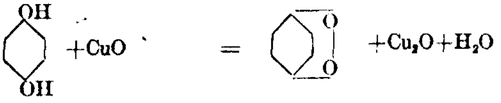


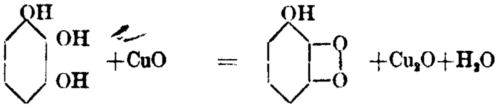
Para trioxybenzene.
Phloroglucin.

The trioxybenzenes are also reducers with formation of quinones, the ortho form being here the most active. Pyrogallol therefore is used as a developer in photography, a case where an active reducer is needed.

Pyrogallol reduces even more actively than hydrokinone, and is much used in making determinations of oxygen, by measuring the diminution in volume of a gas, caused by shaking it in an alkaline solution of pyrogallol.

Both hydrokinone and pyrogallol will reduce Fehling's solution.



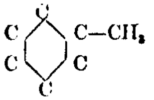


It is evident, therefore, that in their reducing power these di and trioxybenzenes possess the characteristics of aldehydes.

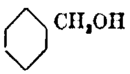
Phloroglucin with HCl, added to a solution of pentose and warmed, affords a red color. This is used as a test for pentoses.

Substitution Products of Benzene.

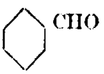
The H of the benzene ring can be replaced (substituted) by various compounds, for instance, methane, ethane, etc., and these can be oxidized in the usual way.



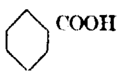
Methan benzene (Toluol), $C_6H_5 \cdot CH_3$.



Methanol benzene (Benzoyl alcohol), $C_6H_5 \cdot CH_2OH$.



Methanal benzene (Benzoyl aldehyde), $C_6H_5 \cdot CHO$.



Methan acid benzene (Benzoic acid), $C_6H_5 \cdot COOH$.

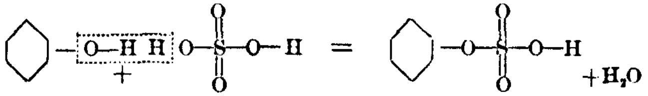
The substitution products of benzene are infinite in number and for further particulars the reader must be referred to the text-books.

Excretion of Benzene Rings.

The cyclic compounds are very stable bodies and cannot be broken up by oxidation in the body. A benzene ring absorbed through the intestine is excreted as such in combination with something else.

1. A phenol (OH) is excreted as an acid ester.

(*a*) In combination with sulphuric acid as a phenol sulphuric acid.



Phenol + sulphuric acid. Phenol sulphuric acid.

It has been said that the OH group of an oxybenzene is slightly acid and has no affinity for acids. This is true in a general way, but the reaction does occur in the body.

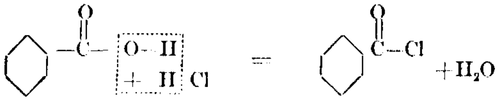
Tyrosin obviously will give Millon's reaction, whereas phenylalanin does not. These compounds will be referred to again.

Now that the constitution of the benzene ring is known, certain reactions, which some of its derivatives give with substances already discussed, may be mentioned.

The principal benzene derivatives used for these reactions are:

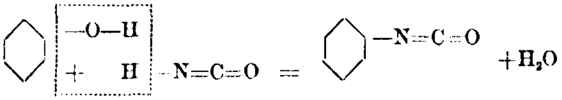
1. Benzoyl chloride.
2. Phenyl isocyanate.
3. Phenyl hydrazin.

1. *Benzoyl chloride* is benzoic acid in which OH is replaced by Cl.



Benzoic acid + Hydrochloric acid, $C_6H_5 \cdot COOH$ — Benzoyl chloride, $C_6H_5 \cdot COCl$

2. *Phenyl isocyanate* is isocyanic acid in combination with phenol.

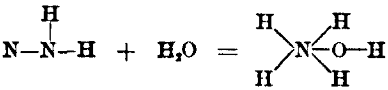


Phenol + isocyanic acid, $C_6 \cdot H_5 \cdot OH + HNCO$ — Phenyl isocyanate, $C_6H_5 \cdot NCO$

3. *Phenylhydrazin.*

To understand the constitution of phenylhydrazin NH_3 must first be referred to.

As a rule when an OH group is attached to N the latter is pentatomic thus:



But one of the H atoms attached to NH_3 may exceptionally be oxidized without N becoming pentatomic.

$$\begin{array}{c}\text{H}\\ |\\ \text{N}-\text{N}-\text{H}\end{array} + \text{O} = \begin{array}{c}\text{H}\\ |\\ \text{H}-\text{N}-\text{O}-\text{H}\end{array}$$

Ammonia. Hydroxylamin.

The reason for calling this an amin is obvious, for if the OH is replaced by $-C \equiv R$ we have one of the ordinary amins with which we are already familiar.

Hydroxylamin may unite with NH_3 with loss of water:

$$\begin{array}{c}\text{H}\\ |\\ \text{H}-\text{N}-\end{array}\boxed{\text{O}-\text{H}+\text{H}}\begin{array}{c}\text{H}\\ |\\ -\text{N}-\text{H}\end{array} = H_2N-NH_2 + H_2O$$

Hydroxyl amin + Ammonia = Hydrazin, N_2H_4.

When an N is attached to another N this is designated by — az —. Thus we have:

$$= N - N = \qquad\qquad - N = N -$$

Azo compound. Diazo compound.

Hydrazin does not occur free but only in combination, mainly with organic compounds — open or closed chains:

Phenol + hydrazin. Phenyl hydrazin.

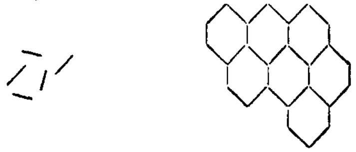
Reactions with Benzoyl Chloride and Phenylisocyanate.

It is much easier to isolate a substance, *i. e.*, to get it in pure form free from impurities, if it is crystallizable rather than amorphous. Crystals are definite bodies with definite melting points, which is seldom the case with amorphous forms.

Substances belonging to the open chain series, as a rule, do not crystallize well, whilst the cyclic compounds readily do so.

If one pictures to oneself a number of single chains lying about, it is difficult to imagine that on drying out they would arrange themselves in any particular order or shape. On the contrary a number of benzene rings might easily be supposed to fit into each other and build up a crystal.

Diagrammatically:



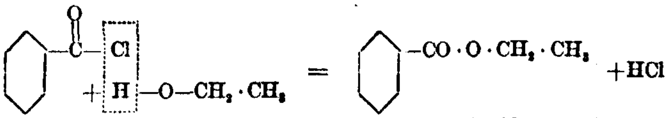
The object then is to find some way of getting a substance which crystallizes badly to crystallize well, or to get a volatile body to crystallize.

By attaching it to a benzene ring a crystallizable substance is produced. This product can be isolated, the benzene ring driven off, and the original substance thus obtained in pure form, the benzene ring merely serving as a medium.

Benzoyl chloride and phenylisocyanate are used as media, and the process is called *benzoylizing*, an expression constantly met with in reading articles on physiological chemistry.

The Cl of benzoyl chloride is only loosely attached and is readily given up to an alcohol OH group to form an ester, or to an NH_2 group to form an amineester.

The reaction occurs in alkaline solution as follows:

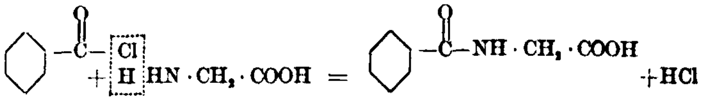


Benzoyl chloride + ethanol, $C_6H_5 \cdot COCl$ = Ethyl benzoyl ester, $C_6H_5 \cdot COO \cdot CH_2 \cdot CH_3$.

The alcohol has been *benzoylized.*

The above is merely given as a simple example of the reaction which occurs with an alcohol. As a matter of fact ethyl-benzoyl ester does not crystallize, but is an oily fluid which, however, is easily separated out. But the polyatomic alcohols, and more particularly the monosaccharides, afford easily crystallizable products with benzoyl chloride.

(2) With amids, amins, and amino acids, benzoyl chloride in alkaline solution forms aminesters.



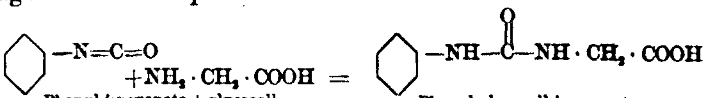
Benzoyl chloride + glycocoll. = Benzoyl glycocoll aminester.

The glycocoll has been *benzoylized.*

In benzoyl glycocoll aminester, hippuric acid will be recognized again. It can therefore be produced in the laboratory as well as in the body.

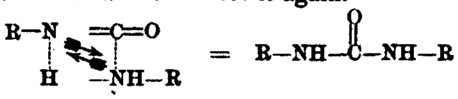
Phenylisocyanate is used for the same purpose as benzoyl chloride. It affords more definite compounds than benzoyl chloride, so is more valuable for accurate work, but its expense prevents its use in the laboratory for ordinary purposes.

The reaction with NH_2 groups may be given, taking glycocoll again as an example.

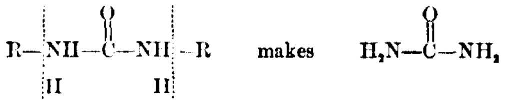


Phenyl isocyanate + glycocoll. = Phenyl glycocoll isocyanate.

It will be observed that in this case there is no loss of water. The C of the $N = C = O$ group detaches one valence from the N to join to the NH_2 group, and the H thus liberated attaches itself to the first N and saturates it again.



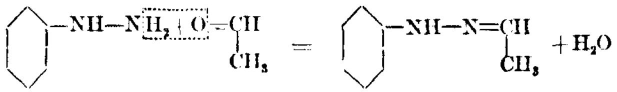
A curious point about the aminisocyanates may be brought out by substituting H for the radical on either side of the isocyan group.



or urea, so that we may regard the isocyanates as merely substituted urea.

PHENYL HYDRAZIN, $H_6C_5 . NH . NH_2$.

The NH_2 very readily gives up its two free H atoms to an aldehyde or ketone group, the H atoms attaching themselves to the aldehyde or ketone O to form H_2O and the N to the C by two valences

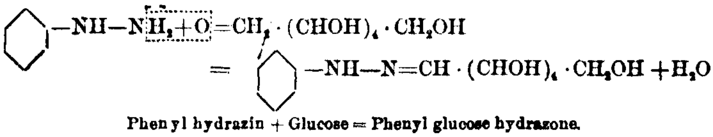


Phenyl hydrazin + ethanal, $C_6H_5 \cdot NH \cdot NH_2$. = Phenyl ethyl hydrazone, $C_6H_5 \cdot NH \cdot N \cdot C_2H_4$.

This reaction is much used as a test for aldehydes and ketones, the resulting hydrazones, as they are called, crystallizing easily.

The osazone test for sugars is based on this reaction but here the process is more complicated.

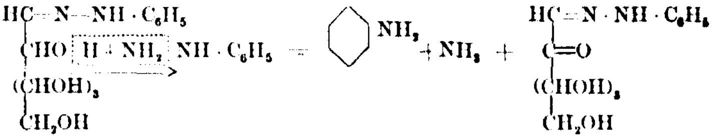
If to a solution of a hexaldose (glucose) phenylhydrazin is added and the mixture warmed, three successive reactions occur.



Phenyl hydrazin + Glucose = Phenyl glucose hydrazone.

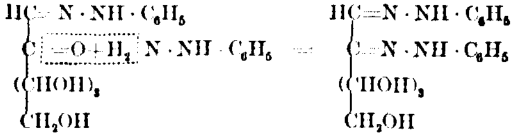
(2) A second molecule of hydrazin now attacks the group lying next to the aldehyde group, depriving it of its two hydro-

gen atoms; at the same time forming anilin with release of NH_3. The CHOH group of the sugar thus attacked has only an O left and becomes a ketone.



Phenyl hydrazin + Phenylglucosehydrazone = Intermediate body.

(3) The ketone thus formed is immediately attacked by a third phenylhydrazin molecule as follows:



Intermediate body + Phenyl glucose hydrazone = Glucosazone (an osazone).

With ketoses *the ketone* C = O *group* is *always next to one of the end* CH_2OH *groups*, so that the ketone group of a ketose may be regarded as holding an alpha position.

Phenylhydrazin brought in contact with a hexketose (fructose) first forms a phenylketosehydrazone with the ketone group.

A second molecule of the phenylhydrazin then deprives the adjacent CH_2OH group of its hydrogen atoms, forming anilin and NH_3.

This newly formed aldehyde group then reacts with a third molecule to form the osazone. Thus, although in this case we start with a ketose (fructose), the final product is precisely the same as with an aldose (*e. g.*, glucose).

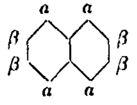
This can be easily verified on working out the reaction, with the help of graphic formulas. In each case a definite crystallizable substance with a definite melting-point is obtained, but

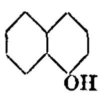
the two substances may be precisely alike, providing their remaining H and OH groups are arranged in the same way. Where these differ the osazones will differ slightly in solubility, so that to a limited extent monosaccharides may be differentiated by means of their osazones.

Double Rings.

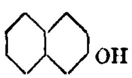
To the benzene ring a second ring may be attached to form naphthalene, and this may be oxidized to naphthol.

Naphthalene, $C_{10}H_8$





α naphthol, $C_{10}H_7OH$



β naphthol, $C_{10}H_7OH$

On the double ring it makes a difference whether the OH group lies next to the link, as the alphas, or one removed from it as the betas. It makes no difference on which of the four alphas the OH is placed. Its relations to the rest of the molecule are the same in each instance, and the same is true of the four beta positions. There are therefore two naphthols, *α* and *β*.

Molisch's Reaction for Sugars.

If an alcoholic solution of *α* naphthol is added to a solution containing sugar, or any carbohydrate, and a little H_2SO_4 then carefully poured down the side of the tube, the latter sinks to the bottom and at the point of junction a violet color at once appears, due to formation of furfurol.

This is a very delicate reaction, occurring even if the sugar is in combination. Proteid material containing glycosamin in combination gives it.

Cholesterin

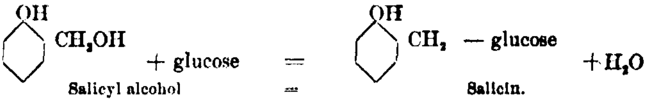
is a fat-like substance, which however is not a true fat, but a higher monoatomic alcohol, the empirical formula of which

is $C_{27}H_{45}OH$. Since there are not enough hydrogen atoms to form a chain it is supposed there must be a benzene ring in it somewhere, but its constitution is not understood.

Glucosides

are glucose combined with some cyclic alcohol to form glucose ethers. Glucosides do not reduce Fehling's solution or form osazones, so it is probable that the aldehyde group enters into the combination. (Cf. Disaccharides, p. 50.)

Example:



Salicyl alcohol = Salicin.

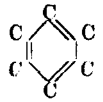
Salicin is found in the bark of the willow.

There are a large number of glucosides, but since they are exclusively products of plant life, never being found in animal tissues, they need not detain us.

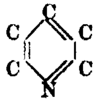
Heterocyclic Compounds.

If the ring or one of the rings contains fewer than six carbon atoms it is irregular and is called heterocyclic.

One of the six C atoms may be replaced by N



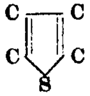
Benzene.



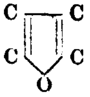
Pyridin.

Or the ring may consist of four C atoms with NH, S, or O. Thus:

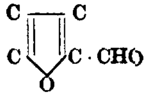
Pyrrol.



Thiophen.

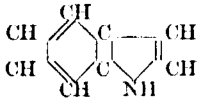


Furan.

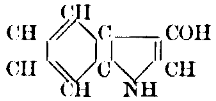


Furfurol.

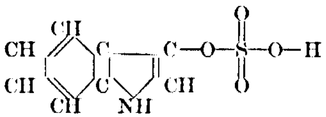
As is the case with the isocyclic, the proteid molecule is poor in heterocyclic compounds. There is in fact only one of importance, a ring of which indol is the best known representative. Indol has a double ring, of which one is irregular.



Indol.



Indoxyl.



Indoxylsulphuric acid (urine indican).

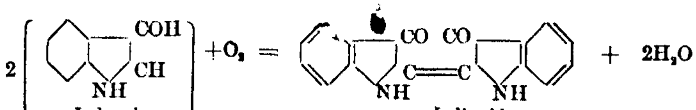
Indol is oxidized to indoxyl in the body and in consequence of the OH group is excreted, like phenol, in combination with sulphuric acid (urine indican).

Urine indican must not be confounded with the indican of the indigo plant which is a glucoside (glucose + indoxyl, p. 91).

Indol is formed in the large intestine, by the action of bacteria, principally coli communis, as a product of the decomposition of the proteid molecule.

Normally the indol, or by far the greater part of it, passes off with the feces, but if there is intestinal obstruction, or an unusual amount of intestinal putrefaction, the indol may be largely absorbed in the body and undergoes the changes mentioned above.

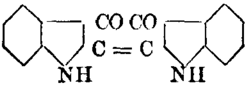
Indoxyl is easily oxidized to indigo blue



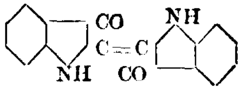
Indoxyl. Indigo blue.

As soon as oxidation occurs one link between the CO and CH is released. Two molecules then combine as above, each losing H_2 which combines with O to form H_2O.

Along with the indigo blue a small amount of indigo red is also formed. Indigo red is the same as indigo blue but with one of the indol rings reversed. The two are space isomers.



Indigo blue.



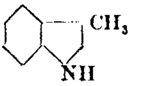
Indigo red.

Indicanuria or indican in the urine can be experimentally produced in animals by resecting a portion of the intestine and replacing it inversely. In this portion peristalsis is reversed, and there is obstruction. Coli communis and other bacteria are able to multiply at any such point, even if it is in the upper part of the small intestine where they do not normally exist.

Indol, therefore, is formed in excess and appears in the urine as indican.

Besides indol, skatol or methyl indol is formed by bacteria in the intestinal tract, and it is to this that the fecal odor is mainly due.

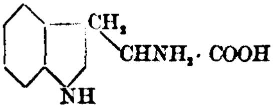
Indol.



Skatol.

Skatol carbonic acid.

Skatol carbonic acid is also a product of proteid decomposition by bacteria.



Tryptophan.

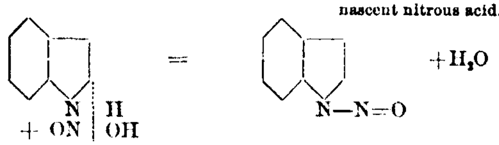
Tryptophan or skatol amino acetic acid, exists in combination in the proteid molecule. (See postscript.)

REACTIONS.

Indol treated with H_2SO_4 and KNO_2 gives a red color, due to the production of nitroso indol.

$$H_2SO_4 + 2KNO_2 = K_2SO_4 + 2HNO_2$$

nascent nitrous acid.



Indol + nitrous acid = Nitroso indol.

Nitroso skatol and the nitroso compounds of the other derivative of indol do not afford the red color.

Tryptophan if free gives a pinkish color with chlorine or bromine water.

Tryptophan in combination in the proteid molecule does not afford this reaction, but it will give a violet color if treated with H_2SO_4 and acetic aid (Adamkievicz reaction).

It has long been known that if the acetic acid has been freshly made it will not give the Adamkievicz reaction. But it has only recently been shown that it is not the acetic acid itself which reacts, but an oxidation product, glyoxylic acid.

$$CH_3\,COOH + O_2 = CHO.\,COOH + H_2O$$

Acetic acid. Ethanal acid (Glyoxylic acid).

Sodium glyoxylate can be easily made by reduction of oxalic acid with sodium amalgam and, since this is stable, it is now used instead of old acetic acid for the test. (Hopkins reaction.)

$$Na.Hg + COOH.COOH = CHO.COONa + H_2O + Hg.$$

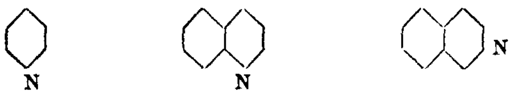
Sodium amalgam. Oxalic acid. Glyoxylic acid.

The Hg merely serves as a restrainer for the Na, which would react too violently if used alone.

On adding H_2SO_4 the Na is driven off and the glyoxylic acid gives the violet color with the tryptophan.

The structure of the colored product is not known.

The *alkaloids* are complicated vegetable products, the constitution of only a few being known. There are three rings which chiefly serve as a basis for their make up.



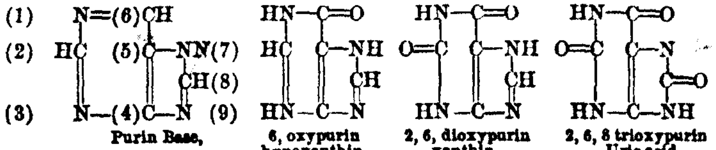
Pyridin. Chinolin. Isochinolin.

The alkaloids are largely poisonous but do not directly concern us except for the fact that at one time they were supposed to be products of plants solely.

If, therefore, alkaloids were found in a body "post-mortem," it was taken for granted that death was caused by vegetable poisoning and many a man has been hanged on this evidence. It is now known, however, that this is an erroneous supposition, for many bacteria produce alkaloids and after decomposition has set in alkaloids may be found in animal organs without death having been necessarily caused by them.

Purin Bases.

Although the purin bases are cyclic compounds they can hardly be classed with those so far under consideration since they are not based on the condensation of ethine.

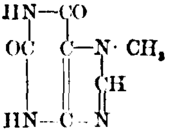


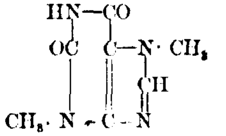
Purin Base, $C_5H_4N_4$. 6, oxypurin hypoxanthin, $C_5H_4N_4O$. 2, 6, dioxypurin xanthin, $C_5H_4N_4O_2$. 2, 6, 8 trioxypurin Uric acid, $C_5H_4H_4O_3$.

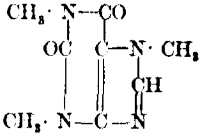
the purin base of which the others are derivatives does not occur in nature, and, until recently synthesized in the laboratory, was a purely hypothetical compound.

The bracketed numbers on the purin base are simply used as a convenient method of indicating which of the central atoms is oxidized or substituted. Thus on speaking of 2,6 dioxypurin we know at once where the oxidation has occurred.

Besides oxidation of the C atoms of the purin base, the N groups may be methylized. Among other derivatives are:

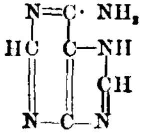

7, Methylxanthin.

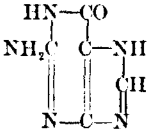

3, 7, dimethylxanthin (Theobromin).


1, 3, 7 trimethylxanthin (Caffein).

Theobromin is the active principle of tea and cocoa whilst caffein is the active principle of coffee.

Purins.— Besides methyl purins, amino purins are known. Of these adenin and guanin are the only ones that need be mentioned.


Adenin, $C_5H_5N_5$


Guanin, $C_5H_5N_5O$

Of the above compounds uric acid is the one which naturally is of the most interest to us. Its graphic formula shows that it contains a central chain C — C — C and two side chains, each NH — CO — NH. If the side chains were split off and H taken up to resaturate the N atoms they would appear as $NH_2 - CO - NH_2$ or urea.

Uric acid is, therefore, closely related to urea, and it was formerly taken to represent an intermediate stage in the oxidation of proteid matter to urea.

This view, however, is now abandoned, and it is thought that the small amount of uric acid occurring normally in the urine and the excess of it in gouty conditions are derived largely from the nucleoproteids, a variety of proteids containing derivatives of the purin base.

This will be treated of more fully in the chapter on proteids, but one of the theories (Hofmeister's) of urea and uric acid production may be discussed here. It seems the most plausible of the numerous theories put forward, but still it is only a theory and must not be taken too seriously.

Urea is the main nitrogenous product of excretion by the kidneys in mammals.

Uric acid is the main nitrogenous product of excretion by the kidneys in birds.

According to Hofmeister, urea is an oxidation product of glycocoll, NH_3 being annexed at the same time.

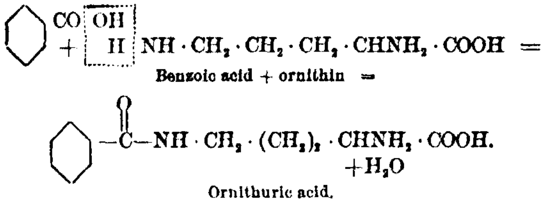
$$\underset{\text{Glycocoll.}}{CH_2NH_2.COOH} + NH_3 + O = \underset{\text{Urea.}}{CO(NH_2)_2} + CO_2 + H_2O$$

Although glycocoll is only found in traces in the body tissue it is obviously available when needed, since, as we have already seen, benzoic acid takes it up to appear in the urine as hippuric acid.

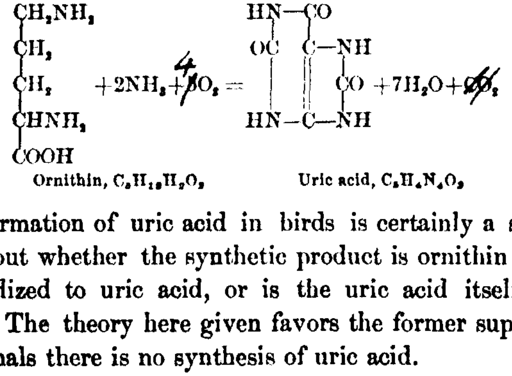
It is known that NH_3 is liberated in the liver, although it does not leave the body as such but as urea. So NH_3 is available.

Oxygen is certainly available. Oxidation is constantly going on in the processes of tissue metabolism. Glycocoll therefore may be regarded as an immediate precursor of urea, the latter being derived from it by oxidation.

If benzoic acid is fed to birds it does not appear in the urine as hippuric acid (aminester of glycocoll), but as an aminester of the diamino acid, ornithin.



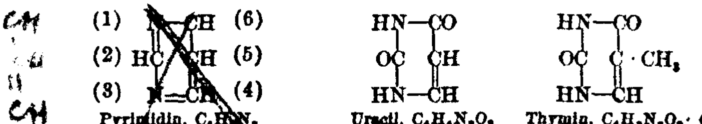
It may be then that ornithin is a precursor of uric acid in birds, even as glycocoll is of urea in mammals. Like glycocoll it may take up NH_3 and O and lose H, at the same time condensing to form a cyclic compound.



Ornithin, $C_5H_{12}N_2O_2$ Uric acid, $C_5H_4N_4O_3$

The formation of uric acid in birds is certainly a synthetic process, but whether the synthetic product is ornithin which is then oxidized to uric acid, or is the uric acid itself is not known. The theory here given favors the former supposition. In mammals there is no synthesis of uric acid.

Pyrimidin Bases.—By splitting off one side of the purin base we get pyrimidin.



Pyrimidin, $C_4H_4N_2$ Uracil, $C_4H_4N_2O_2$ Thymin, $C_4H_3N_2O_2 \cdot CH_3$

The CH groups of pyrimidin can be oxidized to uracil, and thymin is methyl uracil.

Uracil and thymin have been found in nucleo proteids.

CHAPTER VI.

THE PROTEIDS.

The term *protoplasm* employed to designate the living matter of plant and animal cells does not mean a definite chemical substance. Any methods which may be used to determine the chemical nature of protoplasm results in its destruction as living matter so that our ideas of it have been derived from its condition after death.

The compounds which are always found under these circumstances and which are the most characteristic of matter which has been alive, are the *proteids.*

These occur in great variety but their elementary composition is fairly constant, carbon 52 per cent., hydrogen 7 per cent., nitrogen 16 per cent., oxygen 22 per cent., sulphur 0.5–2 per cent.

Phosphorus and iron are found in some proteids. The molecule of proteid is exceedingly large when compared with the compounds which have already been studied, and it is impossible to give any graphic formula to represent its make up. On account of this large molecule proteids in solution are nondialyzable. They will not diffuse through parchment.

There are great differences in the behavior of different proteids toward heat, toward acid and alkaline solutions, and also toward solutions of various salts.

These differences are the basis for the classifications that are made:

A. *Albumens.* Coagulated by heat.	*Globulins.*—Soluble in dilute salt solutions, acids, and alkalies. Precipitated by half saturation of ammonium sulphate. *Albumins.* — Readily soluble in neutral fluids, are not precipitated until complete saturation by ammonium sulphate.
B. *Proteoses.* Not coagulated by heat.	*Albumoses.*—Are precipitated by saturation with ammonium sulphate. *Peptones.*—Not precipitated by saturation with ammonium sulphate.
C. *Albumenoids.*	Proteid-like substance, resistant to most reagents, give many of the proteid reactions.
D. *Proteides.*	Substances made up of a proteid molecule combined with some other group, as the hemoglobin of the blood—Hematin + globin (an albumen).

One has to be careful to distinguish between:

Albu*mens* and Albu*mins.*

Pro*teids* and Pro*teides.*

It is unfortunate that words with different significations should so closely resemble each other.

A. Albumens.

1. The coagulation by heat or by the action of strong alcohol, is a characteristic of the albumens. On boiling a solution all

the albumens are coagulated, but many of them coagulate at considerably lower temperatures. Since the temperature required under similar conditions is constant, it is possible to separate albumens by what is called "fractional heat coagulation." This is accomplished by heating an albumen solution slowly until coagulation begins; maintaining it for a considerable time at that temperature, so that all of that particular albumen "fraction" has time to separate out; then filter and repeat the process at a higher temperature. In this manner albumens may be obtained from muscle which coagulate at 47°, 56° and 63°C.

The coagulated albumen has suffered a slight chemical change so that it will no longer or only slightly dissolve in water. It gives however the same proteid reactions as before, showing that its proteid character is not destroyed.

It is sometimes said to be "denaturalized."

2. Similarly albumens may be separated by fractional coagulation by alcohol. Some are coagulated by dilute alcohol, others not until the alcohol is concentrated.

3. A coagulation similar in many ways to that produced by heat may be brought about in certain albumens by the action of enzymes. For example the clotting (coagulation) of the casein of milk by rennet.

Precipitation must be distinguished from coagulation. By the former term is meant the separation of a proteid from its solution because of the addition of certain salts or acids, in the presence of which the proteid is insoluble, but without undergoing alteration of its chemical character or properties. The albumens are precipitated by saturating the fluid in which they are dissolved with ammonium sulphate. This is called *salting out*. The process does not injure them in any way, they are not denaturalized since they readily dissolve again on removing the

excess of ammonium sulphate, so it is a valuable method for isolating them. Other salts, sodium chloride, zinc sulphate, etc., may be used, but ammonium sulphate gives the best results. It ought to be mentioned, however, that alcohol first precipitates and then coagulates albumens after some time has elapsed, so that if the alcohol is filtered off quickly the albumen can be redissolved. We may distinguish:

1. Heat — coagulates at once.
2. Alcohol — precipitates at once and coagulates later.
3. Salts — precipitate but do not coagulate.

Albumens are divided into two groups:

1. Globulins precipitated by 50 per cent. ammonium sulphate.
2. Albumins precipitated by saturated ammonium sulphate.

Those which have been chiefly studied are the globulin and albumin of blood serum and white of egg.

Their differences may be tabulated:

	Distilled Water.	Saturated Sodium Chloride.	Saturated Magnesium Chloride.
Globulin.	Insoluble.	Insoluble.	Insoluble.
Albumin.	Soluble.	Soluble.	Soluble.

Of the globulins three fractions have been distinguished.

1. Fibrinoglobulin precipitated by 25 per cent. ammonium sulphate.
2. Euglobulin precipitated by 32 per cent. ammonium sulphate.
3. Pseudoglobulin precipitated by 50 per cent. ammonium sulphate.

The globulins are crystallized with great difficulty, but of the albumins egg albumin and the serum albumin of the horse

can be readily crystallized by special methods, and thus be obtained in purer form than any of the others for analysis.

B. Proteoses.

These are formed from the albumens as intermediate products of hydrolysis.

Diagrammatically :

By hydrolysis one molecule of an albumen has been split into two molecules of a proteose.

Hydrolysis of albumens can be effected by boiling with dilute acids or by the action of digestive enzymes.

Proteoses, therefore, have a smaller molecule than albumens, and consequently diffuse more rapidly in solution. No doubt this also accounts for their not being coagulated by heat. The larger the molecule the more easily it is denaturalized.

The basis for their separation into albumoses and peptones is their behavior toward ammonium sulphate (see table).

C. Albumenoids.

The albumenoids form the scaffolding of the body. They are not themselves a constituent of protoplasm, but are derived from albumens partially oxidized by the living protoplasm of cells, and thrown off to serve as a framework. The various kinds of connective tissue are albumenoids, and being very resistant to any but the strongest reagents make very suitable coverings for the individual cells (fibrous tissue), and even for the entire body (keratin).

D. Proteides.

This group includes :

1. *Glycoproteids.* — Compounds of albumen loosely combined with glycosamin or other carbohydrates.

2. *Hemoglobins.* — Compounds of hematin with globin (an albumen).

3. *The Nucleoproteids.* — Compounds of nucleinic acid with albumen.

Summarizing the knowledge thus far gained we find :

1. The albumens.
2. Proteoses. — Albumens modified by hydrolysis.
3. Albumenoids. — Albumens *modified* by body cells.
4. Proteides. — Albumens + something else, the albumen imparting the proteid character.

All these are included under the general term proteid, but the albumens are obviously the corner stone of the whole edifice, so are called "true proteids."

In speaking, therefore, in the following pages of the proteid molecule this practically refers to the albumens.

Ultimate Analysis.

The approximate elementary constituents of proteids have already been given. On ignition there is always found a small amount of ash. This varies with the kind and purity of the proteid, but is made up of varying amounts of chlorides, sulphates, phosphates and carbonates of the metals sodium, potassium, calcium, magnesium and iron. Some of this ash is mineral matter merely mixed with the proteid, whilst a portion is probably combined. There is reason to doubt that a truly ash-free proteid has ever been obtained.

Many attempts have been made to determine the molecular weight of albumen and other proteids by means of ultimate

analysis, and by determination of the osmotic pressure of their solutions.

No accurate figures have yet been obtained, but it is certain that the molecule is very large, that there is great variation in its size and that it decreases with the progress of hydrolysis, so that albumoses and peptones have a much smaller molecule than the albumens. Some attempts have been made to arrive at a structural formula for the comparatively simple peptones, but even these are so extremely complex that this has not yet been accomplished.

Ultimate analysis of proteids therefore has been practically abandoned, but by *hydrolyzing* or *oxidizing* the entire molecule of a proteid it can be split up into fragments of a simple nature, whose constitution is known, and it is possible that by a study of these fragments the manner in which they are combined to form the proteid molecule may be learned.

Oxidation by means of potassium permanganate or other oxidizing agents is unsatisfactory because:

1. The oxidizing process cannot be accurately regulated so that results are not constant.

2. The fragments so obtained probably do not exist *preformed as such* in the original molecule.

Hydrolysis, effected by means of boiling with dilute mineral acids, or by treating with digestive enzymes, such as trypsin, at body temperature, is much more satisfactory because:

1. The process can be accurately regulated.

2. The fragments obtained probably exist *preformed as such* in the original molecule.

These fragments combined together go to build up the proteid molecule, so they are called its *nuclei*.

The object is to get out the nuclei as nearly intact as possible. It has been said that albumens on hydrolysis yield the proteoses

as intermediate products. The proteoses are called intermediate products because, on carrying on the hydrolysis still further, they can be split up into the comparatively simple nuclei.

$$\text{Proteid} + n(H_2O) = \text{Proteoses.}$$

$$\text{Proteoses} + n(H_2O) = \text{Nuclei.}$$

We may now briefly consider:

1. Products of hydrolysis.
2. Products of oxidation.
3. Products of bacterial action.
4. Products of body cells.

1. Products of Hydrolysis.

Proteids on hydrolysis have been found to yield the following list of nuclei, though not all of them are found in all proteids. The principal ones only are given.

A. Monoamino Acids.

1. Aminoacetic acid (Glycocoll), $CH_2NH_2 . COOH$.
2. Aminopropionic acid (Alanin C_3), $CH_3 . CHNH_2 . COOH$.
3. α aminocaproic acid (Leucin C_6),

$$CH_3 . (CH_2)_3 . CHNH_2 . COOH.$$

B. Amino Diacids.

4. Amino succinic acid (Asparaginic acid C_4), $COOH.$-$CHNH_2.CH_2.COOH$.
5. Aminoglutaric acid (Glutamic acid C_5), $COOH.CHNH_2 - (CH_2)_2.COOH$.

C. Diamino Acids.

6. Diamino acetic acid, $CH(NH_2)_2.COOH$.
7. α, δ, diamino valerianic acid (Ornithin C_5), CH_2NH_2-$(CH_2)_2.CHNH_2.COOH$.

8. α, ϵ, diamino caproic acid (Lysin C_6), $CH_2NH_2.(CH_2)_3$-$CHNH_2.COOH$.

D. Amino hexose.

9. Glycosamin (C_6) $CHO.CHNH_2.(CHOH)_3.CH_2OH$.

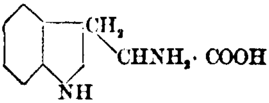
E. Isocyclic compounds.

10. Phenylaminopropionic acid (Phenylalanin C_9), $C_6H_5.CH_2$.-$CHNH_2.COOH$.

11. Paraoxyphenylaminopropionic acid (Tyrosin C_9), C_6H_4-$(OH),.(CH_2.CHNH_2.COOH)$.

F. Heterocyclic Compounds.

12. Skatol amino acetic acid. (Tryptophan.)



G. Unknown Composition.

13. Histidin, $C_6H_9N_3O_2$.

H. Sulphur Compounds.

14. α amino β thio propionic acid (cystein) CH_2SH.-$CHNH_2.COOH$.

All the above are amino acids with the exception of glycosamin (amino aldehyde), but there is another exception of considerable importance.

15. Guanidin, $CNH(NH_2)_2$.

Guanidin occurs in combination with ornithin to form arginin with loss of NH_3, so is not split off on hydrolysis. Arginin is found as the nucleus but is in reality a double (binary) compound, Guanidin + ornithin.

Two points may be noted.

1. The nuclei all contain nitrogen.

2. The NH_2 is always on the C next to the COOH group, *i. e.*, the nuclei are alpha amino acids.

II. Products of Oxidation.

Oxidation carries the decomposition of the proteid molecule much further than hydrolysis, oxalic acid and gases CO_2, NH_3, N and H_2O being among the final products. Intermediate products are more or less complex oxycids about which not much is known. The point to be grasped here is that oxidation attacks the individual nuclei, even though they may be still attached to the main molecule, whilst hydrolysis simply splits up the main molecule, leaving the nuclei intact.

III. Products of the Action of Bacteria.

A third method of attacking the proteid molecule is by means of bacteria. Many bacteria can hydrolyze proteids, *e. g.*, liquefaction of gelatin or blood serum, but their action does not stop here. They have a way of splitting off the CO_2 of the acid group from the amino acids, often leaving the rest of the nucleus intact, for a time at any rate. Ornithin and lysin are often acted on in this way.

$$CH_2NH_2 \cdot (CH_2)_2 \cdot CHNH_2 \cdot COOH = CH_2NH_2 \cdot (CH_2)_2 \cdot CH_2NH_2 + CO_2$$

Diamino valerianic acid (ornithin). — Tetramethylendiamin (putrescin) + carbon dioxide.

$$CH_2NH_2 \cdot (CH_2)_3 \cdot CHNH_2 \cdot COOH = CH_2NH_2 \cdot (CH_2)_3 \cdot CH_2NH_2 + CO_2$$

Diamino caproic acid (lysin). — Pentamethylendiamin (cadaverin) + carbon dioxide.

These and many others formed in a similar way from monoamino acids are the bases which we so often read about as being products of putrefaction.

Cadaverin and putrescin now suggest more to us than merely something with a bad smell.

The bacteria may then proceed to partially oxidize these bases, forming oxyamins.

The bases and their partial oxidation products resulting from the action of bacteria are called *ptomains*.

Instead of CO_2, bacteria may split off NH_2, forming acids instead of amins. Finally the ptomains or other products on further bacterial action are oxidized and broken up into gases and nitrates. These processes are carried on by various groups of bacteria, a fresh group taking up the work where the action of a previous group ceases, these changes occurring naturally in both soil and water.

IV. Products of Action of Body Cells.

Hydrolysis and oxidation go on hand in hand in the body, the final products being urea, CO_2 and H_2O. Intermediate products which do not leave the body as such are called *leucomains*.

The leucomains are analogous to the ptomains.

Summarized in tabular form :

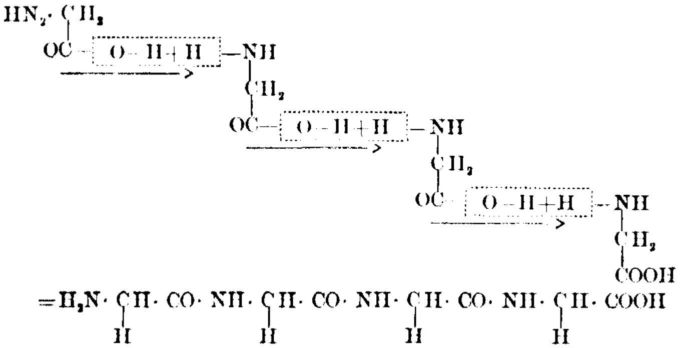
Method.	Process.	Intermediate Products.	Final Products.
Acids or enzymes.	Hydrolysis.	None.[1]	Amino acids, nuclei.
Oxidizing agents.	Oxidation.	Oxyacids.	Oxalic acid, gases, water.
Bacteria.	Hydrolysis + Oxidation.	Ptomains.	Nitrates, gases, water.
Body cells.	Hydrolysis + Oxidation.	Leucomains.	Urea, gases, water.

Or diagrammatically, the numbers indicating various nuclei linked together

[1] The earlier intermediate proteoses are not taken into consideration.

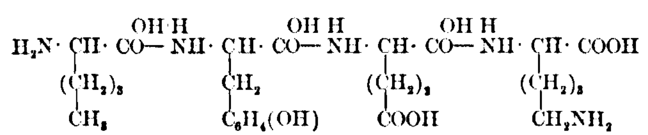
neutralized as well as the COOH groups. In this way we can build up the complex as follows :

2. Combination OC — N.



At one end there is the basic NH_2 and at the other the acid COOH, so that the acid and basic qualities are precisely the same as in the original nuclei, and remain the same, no matter what the size of the complex may be.

This constitutes a glycocoll chain, but remembering that our nuclei are alpha amino acids, R — $CHNH_2$.COOH, we can substitute any of them instead of glycocoll, and in this way build up a part of the proteid complex as follows :



Leucin. Tyrosin. Glutamic acid. Lysin.

The dotted lines show where combination took place and where splitting would occur on hydrolysis.

1. *Hydrolysis.*—The nuclei are split off as such.

2. *Oxidation.*—The side chains may be supposed to become oxidized down until we get

$$H_2N \cdot \underset{|}{CH} \cdot CO{-}NH \cdot \underset{|}{CH} \cdot CO{-}NH \cdot \underset{|}{CH} \cdot CO{-}NH \cdot \underset{|}{CH} \cdot COOH$$
$$COOH \qquad COOH \qquad COOH \qquad COOH$$

The next step would be to split off CO_2 and oxidize the CH groups of the main chain into

$$H_2N \cdot CO \cdot CO \cdot NH \cdot CO \cdot CO \cdot NH \cdot CO \cdot CO \cdot NH \cdot CO \cdot COOH$$

But such a combination would be so unstable that it would hydrolyze of itself, without any stimulus in the shape of a catalyzer such as a mineral acid or trypsin, into $(CONH_2.COOH)_n$ oxalamid.

Oxalamid again is very unstable and would of itself hydrolyze to oxalic acid, COOH.COOH and NH_3.

Oxalic acid, CO_2 and NH_3 are among the chief products of oxidation by potassium permanganate. The fact helps to support the theory.

3. *Hydrolysis + oxidation* as in the body.

Supposing the side chains oxidized down to

$$\begin{array}{c} \qquad\quad OH\,H \qquad\quad OH\,H \qquad\quad OH\,H \\ H_2N \cdot \underset{|}{CH} \cdot CO \,\vdots\, NH \cdot \underset{|}{CH} \cdot CO \,\vdots\, NH \cdot \underset{|}{CH} \cdot CO \,\vdots\, NH \cdot \underset{|}{CH} \cdot COOH \\ COOH \qquad COOH \qquad COOH \qquad COOH \end{array}$$

If hydrolysis occurs at this point, we get $(COOH.CHNH_2.COOH)_n$ or amino malonic acid.

But this is very unstable, being easily, by slight warming even, split up into glycocoll, $CH_2NH_2.COOH$, and CO_2.

As already mentioned glycocoll is very likely the immediate precursor of urea.

There are other arguments which can be used in support of this theory, but they need not be discussed here. It must not, however, be forgotten that this is still a theory. There is no actual proof that the molecule is constructed in this way.

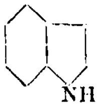
Defects of Hydrolysis.—Hydrolysis certainly affords a better insight into the make-up of the proteid molecule than oxidation, but even hydrolysis is not perfect.

8

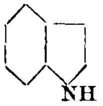
If carried out by means of boiling with acids, certain dark colored condensation products are afforded, so called melanoidins, which certainly do not exist as such in the proteid molecule, and in the formation of which some of the nuclei must be concerned, so to this extent are lost for observation.

On digestion with trypsin melanoidins are not formed, but some of the nuclei may undergo changes.

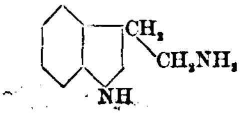
Taking the nucleus represented by the indol ring for example,



It is probable, for reasons given later, that this exists in the proteid molecule as tryptophan. As hydrolysis proceeds the fluid gives a pinkish color on treating with chlorine or bromine water, showing the presence of free tryptophan. But before the hydrolytic process is complete the tryptophan reactions are no longer given. The ring



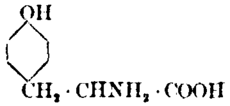
is still there but no longer as tryptophan. It may appear as



skatol methylamin, CO_2 being lost, or in other forms. Again on hydrolysis of proteids either by acids or trypsin NH_3 is given off. This is probably amid N driven off from the aminodiacids. Whatever its origin, it indicates something which is lost for observation.

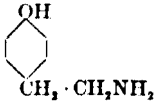
As an example of the different forms which a nucleus may assume according to the way the proteid is treated tyrosin may be taken.

1. Treated with acids, it appears as tyrosin,

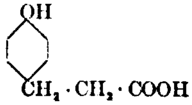


and this is probably the form in which it exists in the proteid molecule.

2. With trypsin some of the tyrosin may lose CO_2 and appear as paraoxyphenylethylamin,

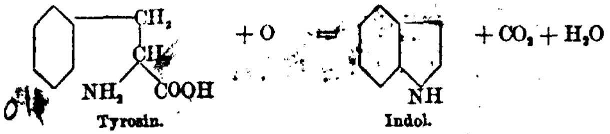


3. With bacteria NH_3 may be lost, and it appears as paraoxyphenylpropionic acid,



4. On oxidation it may appear as phenol or cresol.

5. On fusing dry proteid with KOH there is condensation and indol or skatol is formed.



This shows clearly how close are the relations between tyrosin and indol, but the balance of evidence is in favor of there

being two distinct nuclei in the proteid molecule, one as tyrosin and the other as tryptophan.

TESTS FOR PROTEIDS.

A. *Color Tests.* The color reactions which characterize the proteids are due to certain groups which they contain and not to the molecule as a whole.

1. *Xanthoproteic reaction* (p. 78) is given by all proteids. It indicates the presence of a benzene ring. Either tyrosin, phenylalanin or tryptophan afford this reaction.

2. *Millon's reaction* (p. 77) is afforded by the phenol (OH) group of the tyrosin nucleus.

About equal quantities of Millon's reagent and the proteid solution are mixed and warmed to about 45° C. If allowed to stand for a few minutes the red color appears.

3. *Adamkiewicz reaction* (p. 94) is due to the tryptophan group.

4. *Molisch's reaction* (p. 90) is due to the carbohydrate group.

Note.—Proteid does not reduce Fehling's solution, so the aldehyde group of the glycosamin is probably not free but forms the link which binds the glycosamin to the proteid. (See disaccharides, p. 50.)

5. *Biuret reaction* (p. 60) is due to special combinations of CO groups, together with at least one free NH_2 group. Such combinations are afforded by the nuclei when they are linked together to form complexes, but not by the individual nuclei as such. After complete hydrolysis the biuret test is no longer given, and in digestion experiments this test is applied to determine if the hydrolysis is complete or not.

For this purpose the first four tests would obviously be unsuitable since the substances which afford them as such would still be present.

It is true the tryptophan disappears, but not at any definite point.

B. Alkaloid Reagents. — The proteids are very complex nitrogenous bases and are precipitated by a number of reagents which also precipitate certain simple nitrogenous bases called the alkaloids. For this reason the term alkaloid reagents is applied to them. It is probably the diamino acids in the proteid which give the basis for these reactions. The precipitation is due to chemical reaction, and is not analogous to precipitation by salting out, or to coagulation by heat or alcohol.

The most important of these reagents are:

1. Phosphotungstic acid.
2. Phosphomolybdic acid.
3. Mercury-potassium iodide.
4. Trichloracetic acid.
5. Picric acid.

Nitrogen of the Proteid Molecule.

The nitrogen which is the most conspicuous and characteristic element in proteid is found, not in one or two groups only, but in practically all of the nuclei, so that the peculiar nutritive properties of proteid are not given by one or two groups which overshadow the rest. Of the N about 90 per cent. on an average is in the form of amino acids (stable N) but the remaining 10 per cent. is amid (ammonia) N (p. 61).

It is customary to divide the N (of the hydrolytic cleavage products) into three parts.

(*a*) Amid or ammonia N. Distilled over on boiling with MgO (alkaline base) and the N of the distillate estimated.

(*b*) Acid N, *i. e.*, N of monoamino acids.

(*c*) Basic N, *i. e.*, N of diamino acids.

From the residue of (*a*) the diamino acids are precipitated

by a nitrogen free alkaloid precipitant such as phosphotungstic acid, filtered, and the amount of N in the filtrate, acid N, and precipitate, basic N, respectively estimated. A fourth determination of the total N in the proteid can be made and the percentages of each kind of N arrived at.

Such a determination might come out as follows:

Proteid. Total N 100.

Amid N	Acid N	Basic N
10%	70%	20%

For the following proteids the proportions have been found to be

	Amid NH_3 Nitrogen.	Acid Nitrogen.	Basic Nitrogen.
Crystallized egg albumen.	8 %	68 %	22 %
Hemoglobin.	6	65	24
Casein.	13	76	11

We already know how the amino N comes in, but the question arises: How is the amid N combined? Probably the COOH group of the amino acid enters into combination with the proteid molecules.

$$\underset{\text{Proteid molecule.}}{R}\text{———}\overset{O}{\overset{\|}{C}}\text{—}\underset{\text{Nucleus.}}{R}$$

So that on most of the amino acids no amid N could exist, but some of them are amino diacids and here there might be a spare COOH group for amid N.

$$\underset{\text{Proteid molecule.}}{R\text{———}\overset{O}{\overset{\|}{C}}}\cdot \underset{\text{Asparaginic acid.}}{CHNH_2\cdot CH_2\cdot COOH}+NH_3$$

$$\underset{\text{Proteid molecule.}}{R\text{———}\overset{O}{\overset{\|}{C}}}\cdot \underset{\text{Asparaginic amid.}}{CHNH_2\cdot CH_2\cdot CONH_2}+H_2O$$

Constituents of the Proteid Molecule.

We can now proceed to consider these in detail, but before doing so it must be clearly understood that the nuclei here discussed are not found free as such in fresh animal organs or tissues, or only to a limited extent. We are simply dealing with products of hydrolytic action on tissue or proteids prepared from it.

In normal tissues the amount of proteid being broken down at any given moment of time is so small that the presence of products intermediate between proteid and urea can very rarely be demonstrated.

A. *Monoamino Acids.*

The monoamino acids have an amphoteric reaction. They are acid by virtue of their COOH group and at the same time basic by virtue of their NH_2 group. Since they are by far the most abundant constituents of the proteid molecule they impart this characteristic to it.

The proteid molecule, therefore, is both acid and basic.

3. *Leucin* (isobutylamino acetic acid) is the most important of all the nuclei, being found in all proteids in varying percentages up to 40 per cent. or 50 per cent. This being so it is well to know how it can be obtained from among the products of hydrolysis.

Precipitate the solution with basic lead acetate, to remove the proteid. Filter and remove the excess of lead acetate by passing H_2S through. The black PbS precipitate is filtered off and the filtrate evaporated to small bulk. The process is repeated and after again evaporating to small bulk on the water bath, keep at room temperature for two or three days in an open porcelain dish. As the fluid gradually evaporates the leucin crystallizes out. Under the microscope the crystals appear as globules with a radially striated appearance.

1. *Glycocoll* (amino acetic acid).—Next in importance of the monoamino acids may be ranked glycocoll, not so much on account of its relative abundance among the products of artificial hydrolysis as the fact that it is probably a constant intermediate oxidation product of animal metabolism, the immediate precursor of urea.

Glycocoll is found, as a nucleus, very sparingly in true proteids and may often be wanting altogether, but in the albumenoids, gelatin, collagen, is relatively abundant.

This is significant since the albumenoids are regarded as partially oxidized proteid.

2. *Alanin* (amino propionic acid) is only sparingly present as such but occurs in considerable quantities in combination with phenol to form tyrosin and with indol to form tryptophan. Tyrosin and tryptophan might, therefore, be ranked with arginin and regarded as binary compounds. But the alanin is very firmly attached to them and cannot be separated as such even by drastic methods so it is better to consider tyrosin and tryptophan as definite single nuclei.

B. *Amino Diacids.*

5. *Glutamic acid* occurs more frequently than

4. *Asparaginic Acid.*—It is to one of the COOH groups of diacids that the amid N of the proteid molecule is probably attached, although there is no direct proof of this. It is significant, however, to find that casein, which contains so much amid N (13 per cent. of the total N), also contains a large amount of glutamic acid (10 per cent.).

Reactions.

The monoamino acids and diacids, products of hydrolysis, can be benzoylized; the resulting benzoyl compounds having different solubilities which enable the chemist to separate them

with a considerable degree of sharpness. The solution to be benzoylized should be made strongly alkaline with 10 per cent. NaOH, benzoyl chloride added gradually in small quantities with continuous shaking.

Or the acids may be converted into their ethyl esters and distilled over "in vacuo." Since these esters have different boiling points they may be separated by fractional distillation. There is some difficulty in keeping the pressure at the low point, 4–12 mm., required to prevent decomposition, but, properly carried out, the method gives excellent results.

C. *Diamino acids.*

The simplest of these is 6, diamino acetic acid, which has been found only once as a product of the hydrolysis as casein.

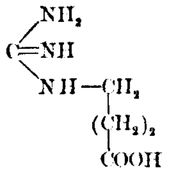
7. *Ornithin* α, δ, diamino valerianic acid, is of much greater interest. It is found among hydrolytic cleavage products combined with guanidin to form arginin. The series of steps by which the structure of arginin was determined forms an interesting chapter in physiological chemistry.

An already mentioned ordinary hydrolysis by acids or trypsin does not split arginin, but by more drastic hydrolytic processes, such as boiling with baryta water, the molecule is split up into urea and ornithin.

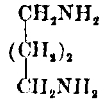
$$\begin{array}{l} NH_2 \\ | \\ C{=}NH \\ | \\ NH{-}CH_2\cdot\ (CH_2)_2\cdot\ CHNH_2\cdot\ COOH + H_2O \\ \qquad\qquad\qquad\quad \textbf{Arginin.} \end{array}$$

$$= \begin{array}{c} NH_2 \\ | \\ C{=}O \\ | \\ NH_2 \\ \textbf{Urea.} \end{array} + \begin{array}{c} CH_2NH_2\cdot\ (CH_2)_2\cdot\ CHNH_2\cdot\ COOH. \\ \textbf{Ornithin.} \end{array}$$

Among the oxidation products of arginin are:



and



Guanidin butyric acid. Putrescin (by bacteria).

Guanidin butyric acid can be further oxidized to:

$$COOH \cdot CH_2 \cdot CH_2 \cdot COOH$$

Succinic acid.

Succinic acid occurs in small quantities in beer among the products of alcoholic fermentation.

It had until recently been supposed that the succinic acid was derived from the carbohydrates of the malt, but it seems more probable that it is an oxidation product of arginin, and therefore derived from the malt proteid.

Arginin is found as a product of hydrolysis in all proteids, being the most abundant of the basic substances. It is especially prominent in the proteid of certain seeds and in the protamins.

8. *Lysin* has been found in seedlings, as a product of tryptic digestion of fibrin, and in certain kinds of cheese. Cadaverin, which stands in the same relation to lysin as putrescin does to ornithin, has also been found in cheese, probably limburger. Both cadaverin and putrescin are constant products of putrefaction.

13. *Histidin* of unknown constitution also occurs among the cleavage products of certain proteids, more particularly of some protamins. The protamins will be discussed later.

	Arginin.	Lysin.	Histidin.
Edestin proteid from hemp seeds,	11.07%	1.3%	1.17%
Proteid from pine seeds,	10.9	.25	.62

These three basic substances:

Arginin	$C_6H_{14}N_4O_2$,
Lysin	$C_6H_{14}N_2O_2$,
Histidin	$C_6H_9N_3O_2$,

have been grouped together to form the hexon bases of Kossel, since each contains six C atoms. Kossel has tried to show that they are closely related to each other and points out the probability that they may be oxidized to hexoses in the body, suggesting that in this way sugar may be derived from proteid. In this, however, he seems to be mistaken since, with the exception of lysin, their constitutions are entirely different from the normal saturated C chains of the hexoses.

Arginin contains five C atoms united to a sixth by means of N, whilst histidin contains too few H atoms to form a normal saturated chain. It must either be an unsaturated compound, or, as seems more probable from recent researches, a cyclic compound allied to the pyrimidins. As a matter of convenience however the term hexon bases may be retained.

The hexon bases are precipitated from acid solutions by the alkaloid reagents, and by this means can be separated as a group from the monoamino acids. The methods for isolating and separating them from each other are too complicated to be given here.

D. *Carbohydrate Nucleus, Glycosamin.*

In speaking of the carbohydrate nucleus of the proteid molecule it must be clearly understood that this is not the only form in which carbohydrates exist in the animal body. Glycogen is stored up in the liver and glucose as such occurs in the tissues apart from the glycosamin in combination.

It has long been known that the proteids contain a carbohy-

drate nucleus, since, among others, Molisch's color reaction indicates the presence of a carbohydrate.

Certain proteids, such as mucin, have been found to yield a relatively large amount of carbohydrates in the earliest stages of hydrolysis with dilute acids, parting with their glycosamin very readily.

These compounds called glycoproteids, are distinguished from the true proteids which yield their carbohydrate group only on complete destruction of the proteid character of the molecule.

The carbohydrate group most frequently found contains nitrogen (glycosamin) and has in many cases been proved to be identical with chitosamin, which has been known for a long time as the chief cleavage product of chitin, the chemical basis of the skeleton of insects and crustaceans.

It is probable that the glycosamin is not present as such in the proteid molecule, but occurs as a dissacharide or polysaccharide, which is broken down on hydrolysis.

The decomposition of nucleoproteids from the pancreas, thymus, spleen, etc., gives a carbohydrate group which has been identified as a true pentose, 1-xylose, and not a lower homologue of the amino sugar glycosamin.

Much of the proof that a carbohydrate group may be derived from a proteid is physiological rather than chemical. For instance, in diabetes large quantities of sugar are excreted in the urine even though no carbohydrate is taken in the food, and when the disease has persisted long enough to remove all the stored carbohydrate and a large portion of the fat. Again, if an animal is starved until the liver is glycogen free and is then fed a pure proteid, glycogen will again appear in the liver.

These instances, however, only show that carbohydrates can be derived from proteid, but the chemist by decomposing a proteid, egg albumin, for instance, and finding glycosamin among

the cleavage products is able to demonstrate its presence as a definite nucleus. There is no evidence to show that animal metabolism can build up sugars from the other constituents of proteids.

AROMATIC NUCLEI.

E. *Isocyclic Nuclei.*

10, Phenylalanin and 11, Tyrosin.

These two closely related compounds are always found among the hydrolytic cleavage products of true proteids. Gelatin, an albumenoid, gives Millon's reaction though as yet no tyrosin has been obtained from it. Gelatin yields phenylalanin on cleavage, but this does not give Millon's reaction. There must therefore, be some phenol group in the gelatin other than tyrosin, or else the tyrosin is combined in some peculiar way that it cannot be obtained as such. Other albumenoids are very rich in tyrosin, keratin sometimes containing as much as 4.5 per cent. The amount of tyrosin found as a cleavage product of different proteids varies very widely.

Egg albumen,	1-2%	Fibrin,	2-3%
Plant albumen,	2	Horn,	3.6-4
Muscle,	1	Casein,	4

Tyrosin separates out with leucin on treatment with lead acetate. It is less soluble than leucin and crystallizes out first in sheaves of needles, reminding one of bundles of wheat. Tyrosin can be separated from leucin by treatment with alcohol to which a little ammonia has been added. In this the tyrosin dissolves but the leucin does not.

Piria's Test. — A drop or two of concentrated H_2SO_4 is added to the tyrosin in a watch glass. After allowing to stand for half an hour to form tyrosin sulphuric acid, dilute the acid with water and neutralize with calcium carbonate. The solution is filtered from the calcium sulphate thus formed and a

drop of neutralized ferric chloride added. A deep violet color appears, probably the iron salt of tyrosin sulphuric acid.

Tyrosin also gives Millon's reaction as already mentioned.

F. *Heterocyclic Nucleus.*

12. Tryptophan has only lately been recognized as a nucleus, so requires a little preliminary explanation.

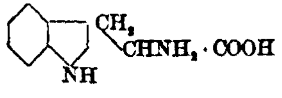
On fusing proteid with KOH, indol and skatol are formed. A part of these are probably condensation products of tyrosin, but more indol and skatol are formed than can be accounted for in this way. Again on decomposition of proteid by bacteria, a large quantity of indol and skatol may be found among the products.

The presence of an indol

ring as a nucleus has therefore been accepted for many years, but until recently there was no evidence to show in what form it exists in the proteid molecule.

The name proteinochromogen or tryptophan was long since adopted for the hydrolytic cleavage product which affords a pinkish color with bromine or chlorine water.

It has recently been found possible to isolate tryptophan by precipitation with mercury bisulphate so that it can be studied in pure condition. This fact has enabled chemists not only to determine its constitution as skatol amino acetic acid



but also to identify it with the substance which affords the Adamkievicz reaction for proteids — violet color with H_2SO_4 and acetic acid.

Before going further three points must be clearly understood:

1. The Adamkievicz reaction indicates tryptophan in combination in the proteid molecule.

2. The bromine or chlorine water reaction is given during the progress of hydrolysis and indicates free tryptophan.

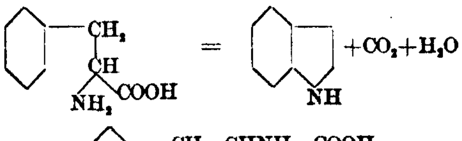
3. About the time that hydrolysis is complete the latter reaction is no longer given. The tryptophan has disappeared.

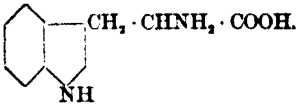
Now the intact proteid molecule gives no reactions for indol or any of its derivatives except tryptophan, so it is supposed in the light of these recent discoveries that tryptophan is the form in which the indol ring exists in the proteid molecule, although tryptophan itself is not found as a final product of hydrolysis.

Like tyrosin the ring may appear in various forms according to the way in which it is treated. With trypsin it may appear as skatol methylamin. On partial oxidation or with bacteria as indol, skatol, or skatol carbonic acid, and in the urine as indoxyl sulphuric acid.

All these may be regarded as derived from tryptophan by splitting off more or less of its side chain.

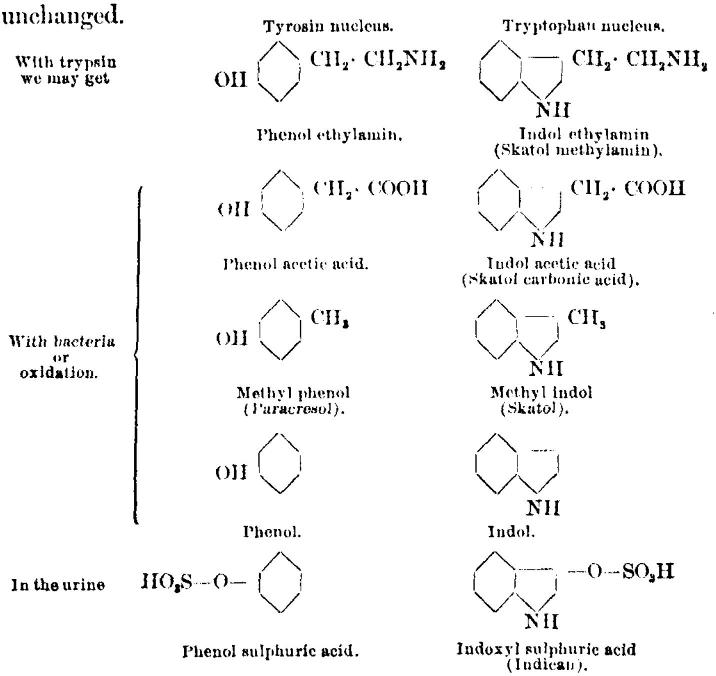
Tryptophan is always called skatol amino acetic acid but if we regard it as indol amino propionic acid (indolalanin) its relations to tyrosin can be much better appreciated.



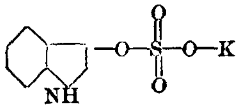


Tyrosin (phenol and alanin) may condense to indol and tryptophan is indol and alanin.

The cleavage products of each are analogous. The alanin, which we may call the side chain, is attacked, but the ring is unchanged.



Indol is a toxic substance and when absorbed in the intestine is carried by the portal vein to the liver where it is oxidized to indoxyl which combines with sulphuric acid to form indoxyl sulphuric acid which is not toxic and is excreted as a potassium salt in the urine.



Skatol is rendered harmless and eliminated in the same way. This synthesis is one of many similar ones designed to protect the organism from the poisonous effects of proteid decomposition products. Phenol and cresol are also eliminated as potassium sulphates.

The amount of combined sulphates in the urine is a measure of the extent of intestinal putrefaction. The sulphates in urine are grouped as mineral (uncombined) and combined, the latter being those in combination with indoxyl or some other aromatic ring.

$BaCl_2$ is added to the urine and this precipitates the uncombined but not the combined sulphates.

$$BaCl_2 + K_2SO_4 = BaSO_4 + 2KCl.$$

The fluid is then filtered and the filtrate boiled with hydrochloric acid. This splits off the indoxyl or other aromatic compound and the combined become uncombined sulphates which can then be precipitated by $BaCl_2$ and weighed as $BaSO_4$.

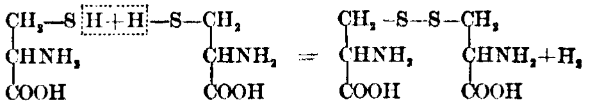
This method gives an index to the total amounts of aromatic products of intestinal putrefaction which are absorbed, indols, phenols, or cresols. A part of these products are excreted in combination with glycuronic acid, but the amounts are comparatively insignificant and are ignored in making the test.

Clinical Test for indican alone. The urine is treated with an oxidizing agent — concentrated HCl containing a little $FeCl_3$; this oxidizes the indican with formation of indigo blue, which is obtained by shaking out with chloroform, in which it is soluble. The depth of blue color in the chloroform solution affords a rough estimate of the amount of indican present.

Indoxyl will oxidize itself to indigo blue by taking up O from the air, but the $FeCl_3$ being an oxidizing agent, hastens the process.

H. *Sulphur Compounds.*

The S of the proteid molecule exists chiefly as cystin. Cystin is a binary compound made up of two molecules of cystein; analogous therefore to arginin.



a amino, β thiopropionic acid Cystein + Cystein = Cystin.

Since the two molecules of cystein combine with loss of H_2, not of H_2O, cystin is not split up by hydrolysis but appears as such among the proteids; not as the ultimate nucleus cystein.

To estimate the total amount of S, the proteid is completely oxidized by nitric acid, $BaCl_2$ added, and the sulphur weighed as $BaSO_4$.

On boiling some proteids with lead acetate in alkaline solution, black lead sulphide, PbS, is quickly precipitated. The alkali splits off the S, which then combines with the Pb. But there is always some sulphur left which cannot be precipitated in this way. The former is loosely combined and the latter is in stable combination.

It was formerly supposed that the loosely combined S exists in unoxidized form R — S — H, and the stable in oxidized form as sulphites or sulphates, so the S of proteids was classified as oxidized and unoxidized.

But there is no evidence to show that oxidized S occurs in the proteid molecule. Sulphites and sulphates are never found among the products of hydrolysis. Moreover experiments with pure cystin have proved that its S is distinctly stable and can only be partially precipitated even by prolonged boiling with alkaline lead acetate.

We may conclude, therefore, that the stable S of the proteid

represents the cystin, whilst the loosely combined must exist in some other form as R — S — H.

Just how it exists is not accurately known, but mercaptans, — methyl, CH_3SH, and ethyl, $CH_3.CH_2SH$ — and thiolactic acid, $CH_3.CHSH.COOH$, have been found as cleavage products on partial oxidation of proteids, though they cannot be regarded as definite nuclei existing preformed in the molecule. In all these compounds the S occurs as R — S — H, and may be split off by boiling with alkalies.

Again a certain amount of H_2S passes off as gas during hydrolysis, and this, since the cystin is not affected, must have been combined in some other form.

The proportions of loosely combined and stable S vary much in different proteids, the former being frequently absent altogether, whilst some of the albumoses contain only loosely combined S.

From some of the albumenoids, the keratins particularly, large amounts of cystin, as well as loosely combined sulphur, have been obtained.

The close relation of cystin to taurin, a constituent of one of the bile acids, suggests a possible origin for the latter compound.

Cystein.	Taurin.
CH_2SH	$CH_2 \cdot SO_3H$
$CHNH_2$	CH_2NH_2
$COOH$	

The transformation has been accomplished in the laboratory as follows:

Cystein.		Cystein sulphuric acid.
CH_2SH		CH_2SO_3H
$CHNH_2$	oxidation by Bromine →	$CHNH_2$
$COOH$		$COOH$

On heating cystein sulphuric acid in a sealed tube CO_2 is split off (so called CO_2 condensation), leaving

$$\begin{array}{l} CH_2SO_3H \\ | \\ CH_2NH_2 \end{array}$$

Taurin.

On feeding animals with cystin it is found that the amount of taurin in the bile is markedly increased. Seeing also how easily the change from cystin can be effected artificially it is considered probable that the taurin of the bile is directly derived from the cystin of the proteid molecule.

Cystin is closely related to serin, a cleavage product of silk fiber.

$$\begin{array}{l} CH_2OH \\ | \\ CHNH_2 \\ | \\ COOH \end{array}$$

α amino β oxypropionic acid. Serin.

Silk fiber is an albumenoid and therefore a modified albumen. Serin is probably modified cystein.

INTERMEDIATE PRODUCTS OF HYDROLYSIS OR PROTEOSES. ALBUMENS MODIFIED BY HYDROLYSIS.

Our discussion so far has been confined to the true proteids or albumens, and their nuclei, or final products of hydrolysis. We have already mentioned, however, that there are certain intermediate products, and these may be taken up more in detail.

A. Acid and Alkali Albumens.

On treating albumen with dilute acid or alkali it gradually undergoes a modification which is not very well understood, but in all probability represents the very earliest stages of hy-

drolytic cleavage. H_2S is given off by alkali, NH_3 by acid treatment; so there must, at any rate, be a change in the molecule of some nature.

The albumen in this condition will act as a base in acid solution, combining with the acid to form a salt. In alkaline solution it acts as an acid, forming a salt with an alkali. In either case the salt is soluble, but on neutralizing the solution the albumen is driven from its combination and precipitates out as a coagulum, showing that it is denaturalized. On again acidifying or alkalinizing the fluid the albumen again dissolves as a salt.

The acid and alkali albumens when in solution are not coagulable by heat but can be easily salted out, resembling globulins in the latter respect. They are very easily hydrolyzed by boiling with dilute acids or by the action of enzymes.

Proteid in this condition may be regarded as intermediate between albumen and the proteoses.

Note. — It must be understood that this change takes place very slowly in the cold, but more rapidly on warming the solution. In speaking, therefore, of proteids in acid or alkaline solution it does not necessarily mean that the proteid is in the form of acid or alkali albumen.

B. Proteoses.

The intermediate products which are obtained when any proteid is subjected to hydrolytic cleavage by any method are called proteoses. This is a general term applied to a large number of substances showing considerable differences among themselves, but which still have enough of the proteid character to distinguish them clearly from the final products of hydrolysis, *i. e.*, the nuclei.

The proteoses as a group resemble the true proteids in that they, speaking very generally, give:

1. All or nearly all the color reactions due to definite nuclei.
2. The biuret test, implying that their internal arrangement is similar.
3. The same chemical precipitation reactions with alkaloid reagents or metallic salts.

They differ from the true proteids in that they:

1. Do not coagulate by heat or by the action of strong alcohol as a rule.
2. Do not as a rule salt out so readily.
3. Are dialyzable and therefore have a smaller molecule.

They are separated from coagulable proteid in solution by boiling and filtering. The filtrate contains the proteoses only.

Since their chemical constitutions are not known they can only be grouped according to their physical properties, and their behavior towards ammonium sulphate is the most convenient method of differentiating them.

Proteoses:
- I. *Albumoses.* Precipitated by saturated ammonium sulphate.
- II. *Peptones.* Not precipitated by ammonium sulphate.

I. *Albumoses.*

A great variety of albumoses has been recognized, but we can only attempt to deal with the more important of them.

Albumoses:
- A. *Primary.* Precipitated by 50 per cent. ammonium sulphate (in acid solution).
- B. *Secondary.* Precipitated by saturated ammonium sulphate.

A. *Primary albumoses* are subdivided into:

Primary albumoses	1. *Heteroalbumose.* Precipitated by saturated *neutral* sodium chloride.
	2. *Protalbumose.* Precipitated by saturated *acid* sodium chloride.

1. *Heteroalbumose* of all the proteoses is the nearest to true proteids, being the only one which is nondialyzable and precipitable in weak alcohol (32 per cent.).

In some respects it resembles the globulins, being insoluble in distilled water, but easily dissolved on adding a little salt.

2. *Protalbumose* is very soluble in distilled water, and is readily dialyzable, so that its molecular weight must be lower than that of heteroalbumose.

It is more soluble in dilute alcohol than in water, but begins to precipitate in 80 per cent. alcohol, although it cannot be completely precipitated except by a mixture of alcohol and ether.

The two primary albumoses can be separated by treatment with weak 32 per cent. alcohol. The filtrate contains the protalbumose only.

It may be remarked that although alcohol will precipitate the primary albumoses it does not coagulate them. They can always be redissolved and in this respect differ from the true proteids. They are not denaturalized even by long standing under alcohol as is the case with albumens.

There is a marked difference in the final cleavage products of the two primary albumoses.

	Heteroalbumose.	Protalbumose.
Leucin,	little,	much.
Tyrosin,	much,	trace.
Glycocoll,	much,	none.
Sulphur loosely combined,	some,	much (1-2%).

Neither of them contains a carbohydrate group.

B. *Secondary Albumoses.*

Three fractions have been distinguished, but it will not be profitable to enter into the details of their differences. More of the true proteid reactions are missing in these than in the primary albumoses, so that in general they represent a further stage of cleavage than the primary albumoses from which they can be obtained by hydrolysis.

It is, however, probable, that on hydrolysis of proteid some of the secondary albumoses are split off direct from the proteid molecule without passing through the primary stage. One in particular called synalbumose contains a carbohydrate group which is wanting in the primary albumoses, so cannot have passed through those stages.

II. *Peptones.*

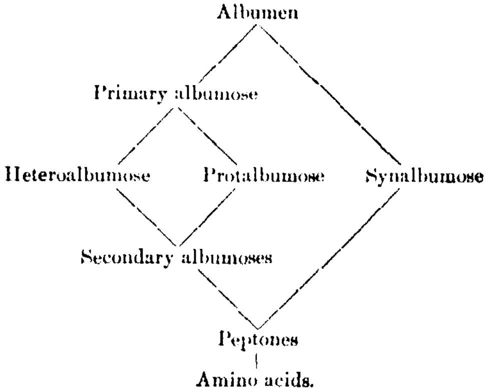
The peptones are the last and simplest of the products of hydrolysis, which still retain some of the proteid characteristics. They are readily dialyzable and cannot be coagulated by heat or precipitated by alcohol or saturated ammonium sulphate.

On the other hand, they can be precipitated by the alkaloid reagents, showing that they contain diamino acids, and they afford the biuret test, so in their internal construction they must still be closely related to the proteids. They are, therefore, recognized as belonging to the proteids, using the term in its broadest sense.

Attempts have been made to classify the peptones according to the ease or difficulty with which they can be hydrolyzed — Kuhne's anti and amphopeptone — but the distinctions drawn are arbitrary and not of sufficient value to discuss in detail.

The little that is known about them indicates that they all closely resemble each other.

One point may be noted, that a proteid at first splits into a number of different albumoses, but on further hydrolysis the latter split into peptones between which only slight differences can be detected.

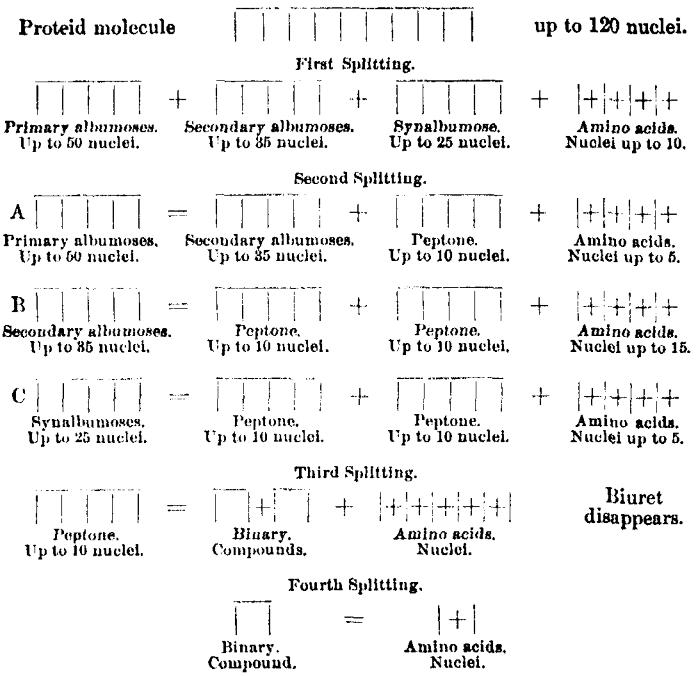


III. *Binary Compounds.*

Between the peptones and the final nuclei there are probably some intermediate binary compounds temporarily present, but only one is known, Leucinimid, composed of two molecules of leucin. Arginin and cystin are binary compounds, but they are not split up by hydrolysis.

No doubt from the commencement of hydrolysis nuclei are being continually split off as such, and the accompanying table gives a hypothetical summary of the probable course of events during the hydrolysis.

The actual number of nuclei in each intermediate product is not known, even approximately, but there are reasons for believing that the nuclei in the original proteid molecule may amount to 100 or 120 in some cases.



We may compare the table with that drawn up for starch in chapter 7, the processes of hydrolysis in each case being analogous.

Polyamino acids.

Proteids — Albumoses — **Peptones** — ***Amino* acids**

Polysaccharides

Starch — **Soluble starch** — **Dextrins** — **Monosaccharides.**

An exact comparison cannot very well be made, since, in some respects, the monosaccharides are more comparable with

the peptones. The monosaccharides are still carbohydrates but the amino acids are no longer proteid. This is a mere matter of nomenclature but the peptones and monosaccharides are the forms in which the proteids and carbohydrates respectively are absorbed and utilized by the body, so that physiologically they are comparable.

Still the amino acids and monosaccharides are the final products of hydrolysis in each case, whilst the peptones and dextrins are intermediate products. By regarding the proteids as polyamino acids and the starches as polysaccharides our method of comparison seems to be justified.

Albumenoids.

The albumenoids in their native condition are insoluble but some of them can be hydrolyzed to soluble forms. The final products of their hydrolysis are the same as those of true proteids. *Collagen* is the chemical basis of white fibrous tissue, forming as such the frame work of the individual organs and tissues, whilst, mixed with lime salts to form the bones, it constitutes the framework of the entire body.

It is resistant to gastric digestion but can be hydrolyzed by trypsin or boiling with dilute acids.

Gelatin is the first intermediate product of its hydrolysis and this stage can be reached by prolonged boiling with water alone. Meat on cooking becomes more tender and this is due to partial hydrolysis of collagen to gelatin. Gelatin itself is not found in the living tissues.

Glue, obtained by boiling bones, cartilage, etc., is simply impure gelatin. The various commercial gelatins are more or less purified glue.

Gelatin in solution, as is well known, dissolves on heating and solidifies (gelatinizes) in the cold. It is supposed by some that the

power of gelatinization depends upon the presence of mineral salts, but the purest gelatin obtainable, containing only .04 per cent. of ash, will gelatinize in 1 per cent. solution so that the rôle of the mineral salts does not appear to be an important one.

Gelatin, although tyrosin cannot be obtained from it, gives the Millon faintly and the biuret reaction very strongly so that it is of a proteid nature, but there appears to be some group or groups lacking which are of importance for nutrition since the nitrogen contained in gelatin cannot entirely take the place of proteid nitrogen.

Gelatin, therefore, is not a proteid food but it is good sparer of nitrogen. In other words if an absolute gelatin diet is given there is less waste of nitrogen from the tissues than with an absolute carbohydrate or fat diet.

Elastin is the basis for elastic fibrous tissue. It is much more resistant than collagen, and requires prolonged action of hydrolytic agents before it is broken down. Both collagen and elastin on hydrolysis afford albumoses and peptones similar to those obtained from ordinary albumen.

Of the final products tyrosin can be obtained from elastin though not from collagen.

Keratin.—The function of keratin is different from that of collagen. It is not a supporting medium but forms the external coverings of the body, horns, hoofs, hair, etc., being secreted by the epithelial cells of the epidermis whilst collagen is secreted by the connective tissue cells.

Keratin is the most resistant of all the albumenoids to hydrolytic agents, and can only be decomposed by boiling with strong acids or alkalis.

The main point of interest is its high sulphur content, 4–5 per cent. Cystin is present in large quantities, its chief source for laboratory experiments or other uses being hoofs or hair.

Albumenoids.	Digestion with		Boiling with		
	Pepsin.	Trypsin.	Water.	Dilute Acids.	Strong Acids.
Collagen.	0	readily	to gelatin	readily	readily
Elastin.	0	slowly	0	slowly	readily
Keratin.	0	0	0	0	slowly

This merely shows the relative speed of hydrolysis.

On boiling with strong acids the intermediate proteoses are quickly decomposed, and the nuclei are decomposed to some extent, so the products cannot be compared with those derived from digestion with trypsin or dilute acids. Cystin, however, is not affected and can be obtained as such from keratin.

Proteides.

There are certain proteids which by mild action of a hydrolytic agent may be split into two parts, one of which is always a complete proteid body, the other being organic but not a proteid. This is analogous to the cleavage of the glucosides into two atom groups one of which is always a sugar. Hoppe-Seyler in consideration of the analogy with the glucosides has designated the complex albuminous bodies the *proteides*. By English authors they are generally called compound proteids. Three classes of these compound proteids may be considered, glyco-proteids, hemoglobins and nucleo-proteids.

Albumen
1. *Glycoproteids*, *Mucins*<
Glycosamin

On cleavage with acids these bodies very readily yield a reducing substance, glycosamin, and proteid. They are viscous colloid substances of a marked acid nature, dissolving readily in dilute alkalies from which solution they are precipitated by acetic acid. The precipitation by acetic acid is used to obtain the mucin from mixed solutions.

They are not coagulated by heat but are denaturalized by long-continued action of acids, alkalies or alcohol. Their elementary analysis shows considerable difference from the true proteid, the difference being due to the large amount of glycosamin, which contains less N and more O than true proteid.

	C.	H.	N.	S.	O.
Mucin	48	6.5	11.5	.8	32 per cent.
Proteid	52	7	16	.5	22 per cent.

Certain mucins have been found to yield as much as 30 per cent. of a reducing substance. The nature of the proteid part of the molecule has been neglected for a study of the reducing substance.

Mucoids.

These compounds have a great similarity to the mucins from which they are distinguished, not sharply, by certain physical properties. They are less slimy than mucins, but the difference between mucins and mucoids are only of degree not of kind.

The name mucin is now restricted to the slimy substances secreted by epithelium; all other compounds having a similar behavior being called mucoids.

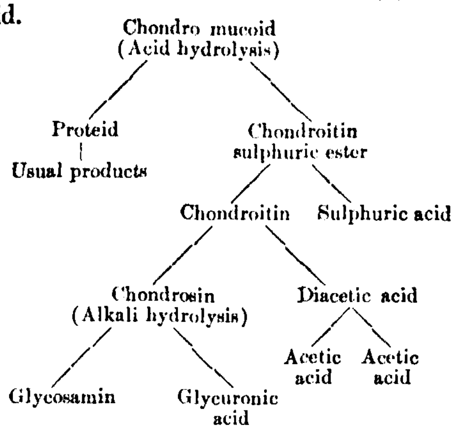
Mucoids have been obtained from tendon, umbilical cord, white of eggs, blood serum, ascitic fluid and cartilage. They are all easily hydrolyzed with the direct release of glycosamin except in one instance, chondromucoid from cartilage, which has certain characteristic cleavage products distinguishing it from all other mucoids.

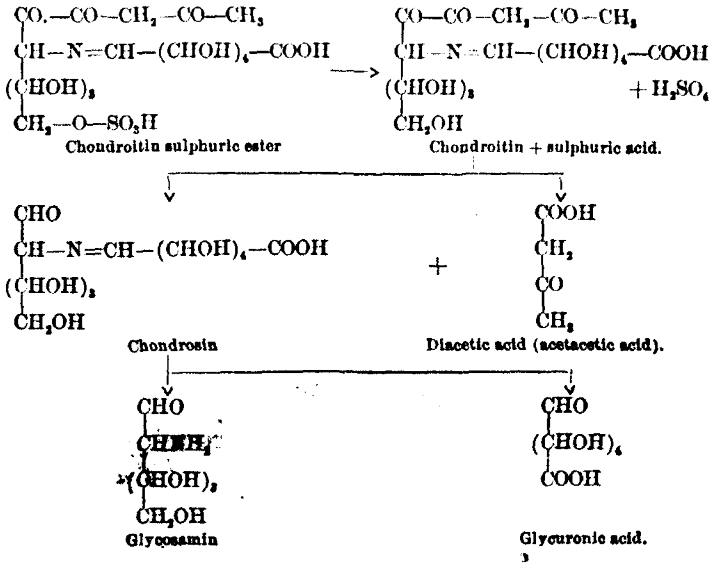
When chondromucin is boiled with dilute acids there appears, in addition to the usual cleavage products of proteid, a sulphur containing chondroitin.

By further cleavage with dilute hydrochloric acid all the sulphur is split off as sulphuric acid, showing that the original compound was in the form of chondroitin sulphuric acid. Chondroitin has no reducing action, but on further cleavage

affords a reducing body, chondrosin, and diacetic acid, the latter being at once split up into two molecules of acetic acid.

Chondrosin on cleavage with alkali yields glycosamin and glycuronic acid.





Chondroitin sulphuric acid therefore is glycosamin with various atom groups attached which can be split off one by one.

Phosphoglycoproteids. — The mucins and mucoids already considered do not contain phosphorus and in this respect differ from the phospho-glycoproteids. The latter on gastric digestion yield a reducing carbohydrate and a phosphorized proteid group. They are of little importance, being only found in invertebrate animals.

II. *Hemoglobin* < Globin, Hematin

Hemoglobin (Hb) is the red coloring matter found in the blood of all vertebrates. It consists of a proteid, globin, combined with a non-proteid iron-containing pigment.

Globin is a member of a peculiar group of proteids called histons which will be described later.

The precipitation and color reactions of hemoglobin are those common to proteids, but it differs in two respects from all other proteids.

1. It crystallizes easily.

2. It has the property of forming loose compounds with oxygen, carbon monoxide, nitric oxide and some other gases.

It is present in the red blood corpuscles as a loose compound with some other constituent of the cell. The chief method of distinguishing the varieties of Hb and its derivatives is by means of the spectroscope. Since space does not permit us to deal with spectrum analysis, we can only touch lightly on the hemoglobins.

It is necessary to distinguish between oxyhemoglobin (HbO) and reduced hemoglobin (Hb). The blood in the lungs is saturated with oxygen, HbO is formed and in the course of the circulation oxygen is given up again to the tissues, leaving the

Hb in the reduced condition. The reduced Hb solution is very dark colored (venous blood), whilst HbO is bright red (arterial blood).

The amount of oxygen which combines with the Hb is not constant but varies with the pressure of the oxygen gas. In this respect it behaves much as gases in aqueous solution. By exhausting blood under the bell jar of an air pump all the oxygen may be removed.

HbO crystallizes more easily than Hb, and is therefore more readily obtained in pure form. Being thus by far the most easily crystallizable of all proteids it has been the favorite one used in molecular weight determinations. The empirical formula has been approximately determined as $C_{758}H_{1203}N_{195}O_{218}FeS_3$ for dog's hemoglobin.

METHEMOGLOBIN.

In addition to the labile oxyhemoglobin, there is another oxygen compound of hemoglobin. This, methemoglobin, is stable in that it will not give up its oxygen in a vacuum. A great many substances have the power of inducing the formation of this compound, and it is often formed in the blood vessels by the action of poisons. In this condition the oxygen cannot be utilized by the body cells.

CARBON MONOXIDE HEMOGLOBIN, HbCO

Is formed when carbon monoxide comes in contact with hemoglobin. It is a comparatively stable substance; the gas is given off in a vacuum very slowly. If the carbon monoxide is present in the air it combines with the hemoglobin to the exclusion of the oxygen because it has a greater affinity for and forms a more stable compound with it. The tissues die for lack of oxygen. The great danger in gas poisoning at the

present day is due to the fact that a large percentage of the gas furnished is carbon monoxide.

Hemoglobin forms compounds with many other gases, but they are not of sufficient importance to describe here.

HEMATIN.

Hematin is the non-proteid iron-containing pigment obtained by cleavage of hemoglobin, a change which takes place very quickly in acid solution. If oxygen is excluded during the cleavage hemochromogen and not hematin results. Hemochromogen may be considered as reduced hematin, the former being a *ferro* compound while the latter is *ferri*. The oxidation and reduction of this substance is accomplished almost as readily as with hemoglobin, and there are reasons for believing that the latter is an ester of the acid hematin with the basic globin.

Hematin itself does not crystallize, but chlorhematin obtained by the action of HCl, by which Cl is substituted for an OH group, crystallizes readily and has been the subject of much study.

$$\underset{\text{Hematin.}}{C_{32}H_{32}N_4FeO_4} + HCl \longrightarrow \underset{\text{Hemin or chlorhematin.}}{C_{32}H_{31}ClN_4FeO_3} + H_2O$$

The hemin crystals obtained from different animal species are to some extent characteristic.

Hematin on cleavage with acids, *e. g.* HBr, yields hematoporphyrin, a pigment containing no iron, which has been shown to be isomeric with one of the bile pigments, bilirubin. *Hematoporphyrin* is much darker than hematin. The bright red color of the latter is due to the Fe.

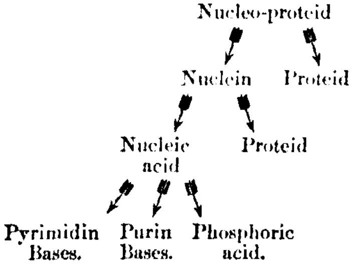
$$\underset{\text{Hematin.}}{C_{32}H_{32}N_4FeO_4} + 2H_2O + 2HBr = \underset{\text{Hematoporphyrin.}}{2C_{16}H_{18}N_2O_3} + FeBr_2 + H_2$$

It is of interest to note that chlorophyll, the iron-containing respiratory pigments of plants, yields a similar compound, phyl-

loporphyrin, on cleavage with acids. The other bile pigments are derived from bilirubin by oxidation and reduction processes.

III. Nucleo-proteids.

Nucleo-proteids are the chief constituent of cell nuclei and are therefore most abundant in glandular organs. They are compounds of proteid with nucleic acid, and the generally accepted view of their decomposition is as follows.



All nucleo proteids do not pass through the exact number of steps given above, and this scheme applies only in a general way. The proteid part of the molecule, as well as the nucleic acid, shows great variation. But a characteristic of these compounds is the high percentage of phosphorus.

C.	H.	N.	P.	S.	O.
48.5	7	17	1.5 %	.7	24.

When subjected to gastric (pepsin + .2 per cent. HCl) digestion, proteid is split off and hydrolyzed to soluble albumoses and peptones, but the residue, (nuclein), containing all the phosphorus, remains undissolved.

Nuclein, therefore, is much richer in P than the original nucleo-proteid, containing 3–5 per cent.

Gastric juice has little effect upon nuclein, but in the intestine the digestion is completed.

All the P of the original molecule is found in the nucleic acids of which the elementary percentages are :

C 37%, H 4%, N 15%, O 34%, P 10%.

Nucleic acid can be dissolved in strong acids, and if to such a solution albumose is added, precipitates are formed which show a remarkable similarity to nucleo-proteids, indicating that proteid, or its modifications, has a strong affinity for nucleic acid.

On further digestion with trypsin or on boiling with strong (5 per cent. HCl) acid, nucleic acid is broken up into its final products, phosphoric acid and the soluble purin bases.

The nucleo-proteids differ to some extent according to the different organs from which they are obtained and some of them yield pyrimidin bases also, — thymin, uracil, — but these are of much less importance than the purin bases and need not be considered. The different nucleic acids yield varying proportions of the following:

Adenin	$C_5H_5N_5$	
Guanin	$C_5H_5N_5O$	also called Xanthin bases or
Hypoxanthin	$C_5H_4N_4O$	Alloxuric bases.
Xanthin	$C_5H_4N_4O_2$	

The simplest method of obtaining the purin bases from the products of proteolysis is by precipitating them with silver nitrate, $AgNO_3$, from the solution made strongly alkaline with ammonia.

$AgNO_3 + NH_3 + H -$ purin base $= NH_4NO_3 + Ag -$ purin base.

The silver compound thus formed is decomposed by H_2S, forming insoluble AgS, and setting the bases free.

Uric acid, $C_5H_4N_4O_3$, trioxypurin, is an oxidation product of these bases and it has been found that in the course of

metabolism in the human body a portion of the nuclein waste is excreted as uric acid.

Such a decomposition as that figured above for nucleo-proteids, resulting in purin bases, takes place in the body tissues, and it has been proved that certain organs, liver and thymus, can oxidize a portion of these to uric acid. The method of demonstrating this is of interest. The liver is taken from the animal at the moment of death, ground to pulp as quickly as possible, nuclein or purin bases added, and the whole kept at 40° C. In three or four hours uric acid is found to be present. It seems probable that all tissues take part in such a formation of uric acid in proportion to their content of nuclein. The nuclein bases taken in the food suffer the same fate as those originating from destruction of body tissue and accordingly the total uric acid excreted may be divided into two protocols.

The *Endogenous*, that part which comes from the purin bases of body tissue.

The *Exogenous*, that portion which comes from the purin bases of the food.

In digestion the nucleo-proteids are split up by pepsin in the stomach to proteid + nuclein. The nuclein is then decomposed by trypsin to nucleic acid + proteid in the small intestine, and the nucleic acid to the purin bases which are absorbed as such ; little or none passing out with the feces.

With an ordinary meat diet the purin bases absorbed are in excess of the needs of the organism, the excess representing the exogenous portion of the uric acid.

When the diet is regulated so that no purin bases are taken in with the food the uric acid output is materially lessened.

Much of the experimental work on the production and destruction of uric acid in the animal body has been confusing because different species of animals used by different investigators meta-

bolize proteid in dissimilar ways. Obviously it is impossible to gain exact knowledge of uric acid metabolism in man by a study of birds because the latter excrete practically all of their waste nitrogen as uric acid, which calls for a synthetic formation of the same whilst in man and other mammals such a process is probably of little importance.

It has been stated above that uric acid may be formed in the liver by simple oxidation of xanthin or hypoxanthin but this statement tells only half the truth, for the process continues and results in a destruction of a portion, at least, of the uric acid thus formed. There are thus two processes taking place simultaneously in this organ and in one animal species the formation, whilst in another the destruction of uric acid is the predominant action.

For instance, if a dog's liver is removed immediately after the death of the animal, tubes inserted in the hepatic artery and vein so that defibrinated blood may be passed through and the whole kept at a temperature of 38° C. the organ is surviving and shows its normal activities. By carefully oxygenating the blood supply the organ may be kept alive for some hours. Such perfusion experiments have proved that uric acid added to the arterial blood is oxidized (to urea) by the liver cells. The cells of other tissues, muscles, kidneys, etc., do the same, so that not all the uric acid formed in the course of metabolism is excreted as such and in a general way we may say that the uric acid excreted represents the portion which has escaped oxidation in the body.

Besides the breaking down there is constant building up by cells to repair waste. Cell metabolism maintains the necessary equilibrium.

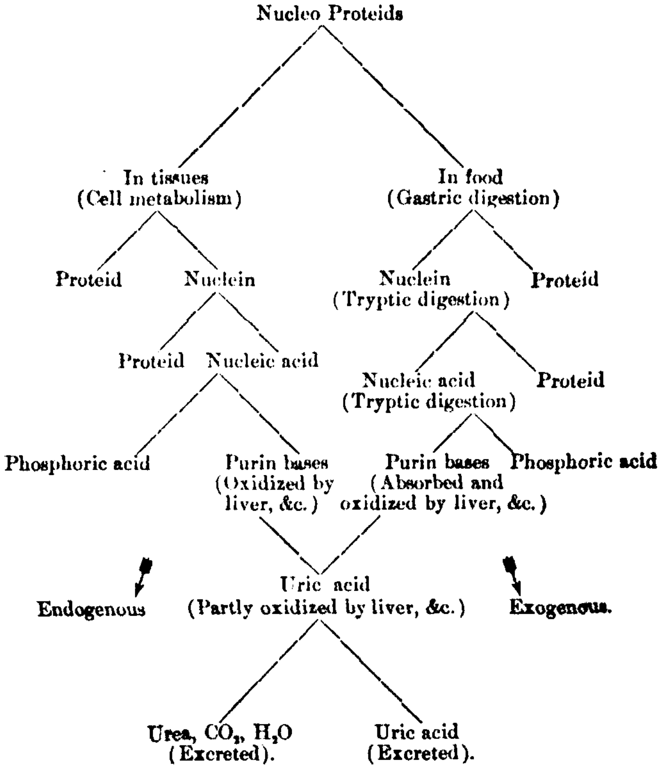
We may therefore trace three sources for urea, two of which have been dealt with previously.

1. Glycocoll, obtained by oxidation of the ordinary proteid nuclei.

2. Guanidin hydrolyzed from its combination with ornithin.

3. Uric acid derived from nucleic acid.

We may again point out that uric acid is not always a precursor of urea as was formerly supposed.



Other Proteids.

We have so far considered typical proteids, the albumens, and their modifications, but there are some proteids adapted for special purposes, which have not the character of albumens. They appear to be closely related to the albumens but are not modifications of them in the sense in which proteoses and albumenoids are regarded as modifications, so will be discussed as separate groups. One can think of them as specialized albumens.

I. *Phosphorized Proteid.*

Casein, the principal milk proteid, is the most important representative of this group.

Casein does not coagulate on boiling but its nutritive properties are impaired, and it is found that some sulphur is split off, so it must undergo some chemical changes. In addition,

1. It contains no carbohydrate group.
2. It contains phosphorus.

1. Is of physiological interest. Free sugar exists in milk in the form of lactose, and it is from this that the sucking mammal derives its carbohydrates, so there is no necessity for these being present in the proteid source of nourishment.

On the other hand eggs contain no free sugar and the embryo bird can only derive its carbohydrates from the carbohydrate group combined in the egg albumen.

2. Since casein contains phosphorus it resembles the nucleo-proteids in this respect and is often called a pseudo-nuclein or nucleo-albumen. But nucleic acid is absent, so these terms are misleading and it is better to regard casein simply as a phosphorized proteid. As with the nucleo-proteids the P is present as phosphoric acid in combination, but it is here in direct combination with the proteid, whilst in the nucleo-proteids the P

is a constituent of the nucleic acid, not being found in the split-off proteid.

Casein.
|
Proteid — phosphoric acid.

Nucleo-proteid.
|
Proteid — nucleic acid.
/ \
Purin bases. Phosphoric acid.

Phosphorus is necessary for the suckling and is obviously supplied by means of the casein. In the egg nucleo-proteids are present in the yolk, and afford the phosphorus required by the embryo bird.

Whilst on the subject of phosphorus a slight digression may be made. Phosphorus is required not only for building up the nucleo-proteids of the nuclei, but also for nervous tissue which contains a large amount of P in the form of lecithin, a phosphorized fat occurring in combination with proteid.

II. *Basic Proteids.*

Histons and protamins.

These differ from albumens:

1. In not being coagulable by heat.

2. In being distinctly basic. They have no acid qualities, so do not give the amphoteric reaction of the albumens. This basic character is due to the relatively large quantities of the hexon bases they contain.

They afford most of the color reactions, but contain no carbohydrate group. The biuret reaction is very marked and this is probably due to the comparatively large number of free NH_2 groups.

(*a*) The *histons* on account of their distinctly basic qualities combine more readily with acids than the ordinary albumens, and are therefore specially adapted for the combinations occurring in some of the proteides.

1. Hemoglobin is a histon in combination with the acid hematin.

2. Some of the nucleo-proteids are histon in combination with nucleic acid.

These are the two forms in which histon occurs in animal tissue, and in studying histon the acids must first be split off in either case. But any method which can be employed for this purpose must at the same time bring about a partial hydrolysis of the proteid, so that the histons as obtained for study cannot be regarded as the exact representatives of the original proteid part of the molecule.

For instance, histons are not coagulated by heat but hemoglobin and the histon containing nucleo-proteids are coagulated. In these cases it cannot be the hematin and nucleic acid respectively which are responsible for the coagulation.

The only way of distinguishing the proteid part (histon) of these substances from true albumens is by noting its distinctly basic qualities, and observing that on hydrolysis it yields the hexon bases in larger quantities than usual. There is no reason for supposing that the average histon molecule is smaller than that of the albumens, although they are often assumed to be simpler substances. This is no doubt true of the form in which they are studied, but probably not of that in which they exist in the entire molecule.

In speaking of the proteid of hemoglobin and of the nucleo-proteids as albumens in the earlier part of this chapter this was done for the sake of simplicity and in order to avoid confusion. It is now evident however that albumen and histon are so closely allied that it is difficult to say there is any real difference between them. Histon probably does not vary from true albumens more than albumens may vary among them-

selves. It only varies in a direction which makes a distinction easier, *i. e.*, in being decidedly basic.

(*b*) *Protamins* vary more from the albumens than the histons, being even more strongly basic, and possessing a simpler structure. Most of our knowledge concerning them we owe to Kossel, who considers them as representing the central portion, or proteid nucleus, around which the more complicated albumens are built up. But there is no conclusive evidence to show that this is the case. They are never found as intermediate products of hydrolysis, but exist preformed in fresh tissues from which they can be obtained direct.

The protamins are not widely distributed, their principal source being the spermatozoa of fish.

It is likely that they are specially adapted for fertilizing processes, but this has not been demonstrated.

The chief feature of the protamins is the large amount of arginin they contain, sometimes over 80 per cent., *e. g.*, spermatozoa of salmon (salmin). Since the protamins are nondialyzable the molecule must be large, and it is quite possible that their molecular weight is not much below that of albumen, but being built up of fewer kinds of nuclei, their structure may be considered simpler in this respect.

Applying the symbol used for the proteid molecule, we may suppose that of each ten nuclei in salmin protein, eight are arginin.

We may suppose the linkage to occur as usual by means of the alpha-amino acid groups, so that for each additional molecule of arginin there would be an increase in basic qualities due to the free guanidin end of the chain. The protamin may be considered as polyarginin to the extent of 80 per cent., whilst

the average albumen is polyleucin to the extent of 40 per cent. or 50 per cent.

To separate protamins from other proteids the solution is boiled. This disposes of the coagulable proteids, and the protamins can be separated from other non-coagulable proteid by NH_3. They exist as soluble salts in combination with an acid, and the NH_3 drives them from combination, forming NH_4 salts. The protamins thus liberated are insoluble and precipitate out.

Unlike the histons, the protamins can be obtained in their native condition. No hydrolytic action is necessary to drive them from a special combination.

The protamins on hydrolysis yield intermediate products called protons. These appear to be somewhat different from the proteoses, but the differences are probably simply due to the relatively large quantities of arginin.

A table of hexon bases found in various proteids may be given for comparison.

	Arginin.	Lysin.	Histidin.
Salmin. Protamin from salmon sperm.	84	0	0
Sturin. Protamin from sturgeon sperm.	58	12	12
Histon from thymus.	14.5	7.5	1.2
Gelatin.	9	5	trace
Casein.	4.5	2	2.5
Egg albumen.	0.8	trace	0

III. *Melanins. Pigments.*

The group tryptophan or proteinochromogen which has recently been identified as skatolamino-acetic acid, has long been considered to be the precursor of animal pigments.

Melanoidin is the term applied to pigments obtained on artificial hydrolysis with acids whilst *melanin* applies to the natural pigments of the skin, hair and retina. They are both very resistant bodies and in origin probably have no relation to

the blood coloring substance hematin. The high sulphur content of many of them does not accord with such an origin, nor do the greater part of them contain any iron.

Melanoidins.—The structure of those coloring substances which are obtained by boiling proteids with mineral acids is very complex and most of the work done thus far has been in the line of ultimate analysis. Such work has not been without some results, for it has established that these "melanoidins" as they are called have wide variations in composition. But the one important fact in regard to structure is that on fusion with caustic potash they yield indol and skatol which harmonizes with the tryptophan idea of origin.

Melanins.—The difficulty of getting the melanins in pure form has hindered a study of their structure but so far as this has been possible, elementary analysis shows marked differences in composition. Fusion with potash yields indol and skatol bodies as with the melanoidins, but until more complete studies are made of their decomposition products it is of little use to say more about them.

On fusion with potash indol and skatol may be formed by condensation of tyrosin as well as from tryptophan and some pigments appear to be directly derived from tyrosin by a process of oxidation. The liquid resembling ink found in the squids has been shown to be such an oxidation product of tyrosin.

Since the melanins are absolutely resistant to hydrolysis, it is impossible, as with albumens or protamins, to find out of what nuclei they are composed. About all we know is that fusion of a very minute amount with KOH gives rise to very bad smells, of skatol and indol, so that in all probability the tryptophan or tyrosin rings or both are very largely represented. Taking them at about 70 per cent., and using our

symbol for the last time we may suppose the melanins to be constructed somewhat as follows:

Tryptophan. Tyrosin. Leucin. Tyrosin. Tryptophan. Glutamic acid.

With the ink of squids and some vegetable pigments the entire molecule appears to be constructed of tyrosin or some oxidized form of tyrosin so that these may be considered as polytyrosins.

Our contention that these groups of proteids just discussed are specialized albumens seems to be justified.

1. *Casein* is especially adapted for sucklings in containing phosphorus and in being easily digested. Since no carbohydrate group is required, this is dispensed with.

2. *Basic Group.*

(*a*) *Histons.* — Specially adapted for combination with certain acid radicals which are required for definite purposes.

(*b*) *Protamins*, probably specially adapted for fertilization.

3. *Melanins.* — Resistant bodies specially adapted for absorption of heat and light rays. This property of the melanins cannot be discussed here, since it entails the question of spectrum analysis.

We have said there are reasons for supposing that the proteid molecule may be composed of 100 to 120 nuclei. To close the chapter we may explain those reasons briefly.

The molecular weight of proteid is difficult to estimate for various reasons, not the least important being the difficulty of getting a proteid in pure condition.

This however does not apply so forcibly to hemoglobin which is easily crystallizable. Its molecular weight has been estimated in various ways.

1. *By O absorption,* 100 grams Hb absorbs .2246 grams O_2.

.2246 : 100 : : 32 : *14,250* molecular weight.

2. *Sulphur content* is .43 per cent.

With lead acetate only part of the S is parted with, so there must at any rate be two atoms of S in the molecule. $S_2 = 64$.

.43 : 100 :: 64 : 14,883 molecular weight.

3. Hb ⟨ Hematin 4.2 per cent. molecular weight is 592. / Globin.

4.2 : 100 :: 592 : *14,100* molecular weight.

4. Estimation from Fe content works out at about 16,000. These estimations agree fairly closely, so we may take the average at 15,000. Deducting 600 for hematin leaves 14,400 for the molecular weight of the globin.

The average molecular weight of the nuclei is about 140, and each time a nucleus enters into combination there is loss of H_2O, having a molecular weight of 18. We may then take the average nucleus in combination as 120 molecular weight.

14,400 ÷ 120 = 120; about the probable number of nuclei in the proteid molecule in this instance at any rate.

TABLE OF PROTEIDS.

1. *Albumen.*
2. *Albumen* modified by digestion.
   Proteoses ⟨ Albumoses / Peptones
3. *Albumen* modified by body cells.
   Albumenoids ⟨ Collagen / Elastin / Keratin
4. *Albumen* plus something else.
   a. Albumen + glycosamin = Glycoproteids.
   b. " (histon) + hematin = Hemoglobin.
   c. " (sometimes histon) + nucleic acid = Nucleo-proteids.
5. *Albumen* specialized.
   a. Albumen phosphorized, Casein.
   b. Albumen basic. Histon and protamin.
   c. Albumen aromatized.—Melanins.

CHAPTER VII.

ENZYMES.

A WORK on physiological chemistry no matter how elementary cannot be considered complete at the present time without a few words on the subject of Enzymes.

Enzymes are soluble unorganized ferments secreted by cells, both animal and vegetable; their main object being so to prepare the food for the cells that the latter can readily assimilate it. The action occurs quite independently of the life of the secreting cell: it is not a vital process.

Until recently a distinction was always made between organized and unorganized ferments; the latter being the enzymes under consideration, and the former various micro-organisms — bacteria, yeasts and moulds — which were supposed to exert their ferment action solely by means of vital processes. This, however, is a distinction without a difference, since it is now known for certain that micro-organisms also effect ferment actions by means of soluble enzymes which they secrete just as is the case with cells of the higher animals and plants.

The term "vital process" may be objected to as being merely a cloak to cover our ignorance. That is precisely what it is. We can follow up the processes which go on in individual cells or the body as a whole, and explain them as due to purely chemical or physical reactions up to a certain point, but beyond this we know practically nothing.

Our knowledge of what is meant by life is a little more extensive than it was, but even yet is a mere nothing by compari-

son with our ignorance. The cloak covers a little less than it did, but it is the same old cloak still.

It has hitherto been found impossible to isolate enzymes and study them in a pure condition, so that their constitution is unknown, and it is even uncertain if they are of a proteid nature or not, but their functions have been very thoroughly studied and a large number of different kinds are known.

1. *Enzymes are catalyzers.*

An astonishingly small amount of an enzyme can apparently exert its action upon an unlimited amount of a given substance, and emerge with undiminished vigor. It is not used up during the reaction.

Again the enzymes are simply accelerators of reactions which would ultimately take place without their aid. They do not originate the reaction in which they are concerned (p. 10).

2. *The action of enzymes is specific.*

A given enzyme will act on a given substance or group of closely allied substances, and on no other. This rule however is not universal; several exceptions are known.

SUSCEPTIBILITY.

Enzymes are very susceptible to certain external influences. Heat above 65° C. to 70° C. and certain poisons will destroy them, although some substances which are poisonous for a cell are not so for its enzymes.

Chloroform, thymol, toluol for instance will destroy the life of a cell but have little or no effect on enzymes. Mercuric chloride is a poison for both.

Chloroform or thymol are often added to a solution in which the action of enzymes is being studied. They prevent bacterial growth which might vitiate the results, but do not affect the enzymes.

The reserve material stored up in seeds and bulbs consists principally of starch, and as these begin to grow the starch is converted by amylase secreted in special cells into maltose in which form it can be utilized by the newly forming sprouts.

Vegetables taken as food by animals contain large quantities of starch which is attacked by the extracellular amylase of the saliva (ptyalin) and pancreas (amylopsin) and so converted into the soluble disaccharide maltose.

Glycogen or animal starch is stored up in the liver and can also be hydrolyzed to maltose by amylase. Amylase is present in the liver cells and here acts as an intracellular enzyme.

2. *Enzymes Hydrolyzing Disaccharides.*

Disaccharides cannot be assimilated as such, but must be first hydrolyzed to monosaccharides. This is effected by means of enzymes of which the principal are:

(*a*) *Saccharase* (Invertase.) hydrolyzes saccharose (Cane sugar.) to glucose + fructose. (Invert sugar.)

(*b*) *Maltase* hydrolyzes maltose to glucose + glucose.

(*c*) *Lactase* " lactose (Milk sugar.) to glucose + galactose.

(*a*) *Saccharase* or invertase is secreted by the cells of the small intestine and the products of its action on saccharose—glucose and fructose can then be absorbed and assimilated.

(*b*) *Maltase* is also secreted in the small intestine, where it splits up the maltose already prepared by amylase (ptyalin and amylopsin) into two molecules of glucose. Maltase also probably exists in the blood, since maltose which finds its way there in small amounts is changed to glucose. Large amounts of maltose introduced into the circulation appear in the urine unchanged.

(*c*) *Lactase* is apparently not secreted in the intestine or elsewhere in the body, but a great deal of lactose is taken in by sucklings with the mother's milk. It seems probable that

the lactose contained in milk is split up in the intestine by bacteria and thus becomes available for assimilation.

The Bacillus coli communis, which is a normal inhabitant of the large intestine, secretes lactase: Many varieties of coli communis also secrete saccharase and it is possible that a part of the saccharose ingested is rendered available by this means.

B. *Enzymes Hydrolyzing Fats.*

Lipase (λίπος fat) is secreted by the pancreas (steapsin) and is discharged into the duodenum, where it splits the fats into glycerin and a fatty acid.

The fats are emulsified by the juices in the intestine and being thus in a very fine state of division are easily reached by the lipase.

The glycerin is absorbed as such, and the fatty acid, combining with any sodium or potassium present, forms a soluble soap. It is in the double form of glycerin and a soluble soap that the fats reach the cells; once there the fat is built up again. Lipase is also found in most of the body cells as an intracellular enzyme.

C. *Enzymes Hydrolyzing Proteids.*

Proteolytic Enzymes — Proteases. — The proteases break up the complex insoluble, or if soluble non-dialyzable, proteid molecules to peptones which can be absorbed by the intestinal cells, and to some extent the peptones still further to amino acids. Pepsin, secreted in the stomach, and trypsin in the pancreas, are always so fully treated of in works on physiology that they need not be discussed here in detail.

Two additional animal proteolytic enzymes have recently been described. *Erepsin* and *Enterokinase.*

Erepsin is said to be secreted by the cells of the small intestine, its function being to extend the action of trypsin, tryp-

sin according to this idea being unable to carry digestion further than peptones, the erepsin then hydrolyzing the peptones to amino acids.

Enterokinase, secreted by the cells of the intestine is said to activate trypsin, the latter being of itself quite inert at the moment of passing into the intestine.

If a pancreatic fistula is made in a dog the juice passing out can be collected. Such juice is said to have no digestive action on fibrin or other proteids, but it is at once activated on adding a little juice from the small intestine. The latter is said to contain the activating enterokinase. It may be that the trypsin is present in the form of a soluble zymogen. Statements about these new enzymes however must still be accepted with some reserve.

The Clotting Enzymes. Coagulases.

Rennet which clots milk and the fibrin ferment (thrombase) which clots blood, like pepsin and trypsin, are always so fully discussed that they need not be more than mentioned here. The presence of calcium salts seems to be necessary for their action and it is thought that clotting is due to the formation of a calcium salt of the casein or fibrin as the case may be. But this is still uncertain; the exact way in which the coagulases act is not known.

Plasmase is a recent discovery. It is secreted in the stomach and is said to have the property of coagulating albumen and albumoses which are then digested by the proteolytic enzymes. But this requires confirmation.

Oxidizing Enzymes. Oxidases.

It has always been a puzzle why certain substances such as albumen, which are oxidized with great difficulty in the laboratory, can be easily oxidized in the body, whilst on the other

hand certain substances, such as oxalic acid, easily oxidized by the chemist, pass through the body unchanged.

Until recently it was necessary to put these oxidation processes under the heading of "vital processes," but the existence of specific oxidizing enzymes in plants is now proved and it has been sought to show that such occur also in animal tissues: specific enzymes which will oxidize definite substances but allow others to pass by.

The oxidizing enzymes are carriers of oxygen. It is supposed that they yield oxygen to the substances to be oxidized and then instantaneously reoxidize themselves. In this they differ from oxidizing substances such as potassium permanganate and chromic acid, which, once deprived of their O, remain reduced and inert. Some Fe and Mn oxides and salts, however, can yield O and then reconstruct themselves, but their action is slow compared with the oxidizing enzymes which work with *inconceivable rapidity*.

Oxidases have the power of turning guaiac tincture blue, by oxidizing it, and are divided into two main groups according to the differences in their action on it.

1. Those which turn guaiac blue direct, utilizing the $O = O$ of the air for their reconstruction: *Direct Oxidases.*

2. Those which do not turn guaiac blue until the addition of H_2O_2. *Indirect or Peroxidases.*

All protoplasm contains an enzyme, *Catalase*, which has the power of reducing H_2O_2 to H_2O and O. The liberated $O =$ is then used by the peroxidases, but they cannot use $O = O$ of the air to reoxidize themselves. The O must be nascent.

Briefly.

1. Direct Oxidases utilize $O = O$.
2. Peroxidases utilize $O =$.

Plants contain both of the oxidases, but in animal tissues only the latter are present.

Animal peroxidases are never poured out into the digestive tract; their field of action is altogether intracellular.

Animal tissues then will liberate O = from H_2O_2 by means of their catalase, and the question then arises: "Is not the oxidation of the guaiac due simply to the O = liberated by catalytic action on H_2O_2?" That it is not so oxidized but requires a carrier can be shown by means of bacterial cultures.

Take an agar culture of any common bacillus and pour into the tube 2 or 3 c.c. of an emulsion of gum guaiac, and then 1 or 2 c.c. of H_2O_2. There is very active evolution of gas. O= must be given off in great abundance, yet there is no bluing of the guaiac. In 10 or 15 minutes add a little blood. A blue color immediately appears.

The bacteria contain catalase but no peroxidase.

We may take it as probable then that catalase and peroxidase are two separate enzymes; the one reducing and the other transferring the O = to other substances.

It seems legitimate to suppose that the reduction of oxyhemoglobin (HBO) in the body is due to the catalase which thus becomes the medium by which O = is supplied to the tissues; the peroxidase acting as carrier and so assisting oxidation. But we must be very careful not to attach much importance to peroxidase. We know that fats, sugars and proteids are oxidized in the body, and we know that, outside the body at any rate, these are not influenced by peroxidase in the least. All we know about peroxidase is that it will oxidize guaiac, but we have no idea what else it can do.

It has been claimed that apart from the peroxidases enzymes are present in the liver and some other organs, which even

after death will oxidize aldehydes (e. g., salicyl aldehyde) to acids. These have been called aldehydases.

But aldehydes are so greedy for oxygen that the mere presence of O= is sufficient for their oxidation, so it appears superfluous to assume a special oxidizing enzyme for this. O = liberated by catalase is all that is needed for oxidation of aldehydes.

The guaiac test for oxidases is simple and may be briefly described.

Put some lumps of gum guaiac into a test-tube containing alcohol and boil over burner until deep yellow. Filter and add the filtrate to water in a test-tube or porcelain dish to the point of a milky emulsion.

1. Into this put a slice of potato. A deep blue color quickly appears which diffuses around the slice. *Direct oxidases.*

2. Instead of potato put in some blood or a piece of animal tissue minced fine. *No reaction.*

Add a little H_2O_2 and a blue color quickly appears. *Peroxidases.*

This is a classical test for blood. Even if the blood has been dried out for months it will give the reaction.

3. Bubbles appear on the surface of the tissue. *Catalase.*

The H_2O_2 is being reduced to H_2O and O.

If the potato or animal tissue is previously boiled no reaction occurs. Both the catalase and the oxidases have been destroyed.

As has already been said, of the peroxidases we know practically nothing beyond the fact that they exist, but of the direct plant oxidases there are several different kinds which afford various definite reactions, but the classification into two main groups as already given is as much as can be attempted here.

We may however mention tyrosinase.

Certain fungi (*Russula* and *Boletus*), if cut with a knife, turn red or blue and finally black on the cut surface. The coloring is due to a melanin formed by the action of a direct oxidizing enzyme, *tyrosinase* on tyrosin. Both tyrosin and tyrosinase exist free in the plant but only come in contact when the tissues are injured.

The tyrosin is oxidized by the enzyme, and the oxidation products condense into a highly complex melanin. Whether the enzyme causes the condensation as well as the oxidation is not known.

The ink of the squid to which we have already referred is a similar substance also formed by tyrosinase, and it may be that some other animal pigments are produced in the same way, but it is not yet known if this is the case or not.

Tyrosinase extracted from *russula* or *boletus* is often used as a test for tyrosin. The merest trace of tyrosin in a solution can be detected in this way by darkening of the fluid.

Reversing Action of Enzymes.

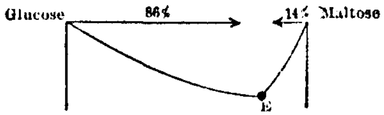
Referring again to the hydrolyzing enzymes we found that they break down complex into simpler substances without being themselves affected. They are catalyzers. But although the enzymes are not used up, yet they are never able entirely to complete the change.

Taking maltase as an example: If this is added to concentrated, 20 per cent., solution of maltose, it will change 86 per cent. into glucose, but leave 14 per cent. unchanged. The percentage varies with the dilution. In a dilute solution a larger percentage is changed but there is always some maltose left. It was formerly thought that the glucose formed acted as a restrainer of the enzyme action but this is not the case, for on

taking a solution in which the action has ceased, and adding more maltose the action begins again, although there is just as much glucose present as before, and this renewed action does not cease until a definite relation is attained between the amount of maltose and glucose.

There is a point of equilibrium (p. 7) beyond which the enzyme is unable to carry the action.

Within the last few years a very curious fact has been ascertained with regard to maltase. If it is added to a concentrated, 40 per cent. solution of pure glucose it *reverses* its action, and starts in to build up maltose. It will do this until the natural equilibrium for this particular concentration is reached, *i. e.*, 14 per cent. of maltose. Then the action stops. There is complete analogy with purely chemical reactions. Constant breaking down of maltose but constant building up at the same rate.



E. Point of equilibrium.

Such a reversing action has been demonstrated for maltase and lipase, but not so far for other enzymes, although it is quite possible they may have the same power. If this is proved to be so it will be a decided step in advance, since it will show that not only breaking down but also building up can be carried on apart from the actual life of the cell. But there are still innumerable reactions taking place in the cell to account for which no enzymes have yet been discovered.

We are for instance quite ignorant to what extent the peroxidases are responsible for oxidation processes. Even if enzymes are discovered for all these reactions there still remains the question: "How are enzymes formed?"

If enzymes are constructive as well as destructive the obvious uses of the intracellular enzymes are enormously extended. They are no longer mere scavengers, intended to break down and clear away what is no longer needed by the cell, but are the agents by which the cell maintains the necessary equilibrium between its protoplasmic contents and those of the surrounding medium, *i. e.*, the extracellular blood or lymph serum.

PLANT ENZYMES.

Among the plants all the enzymes found in animal tissue are represented, with the exception of the fibrin ferment (Thrombase), but on the other hand there are a number of enzymes peculiar to the vegetable kingdom.

Among the more important are:

1. *Cytase* (cellulase), which hydrolyzes cellulose to sugars.

2. *Inulase*, which hydrolyzes inulin (a polysaccharide allied to starch) to sugar.

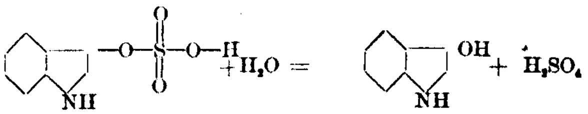
3. *Glucosidases*, usually called glucoside splitters, which hydrolyze glucosides to glucose and something else (p. 91).

There are a large number of different glucosides in plants and a large number of different enzymes which split them. One glucoside and its enzyme may be referred to.

Plant indican is a glucoside and the enzyme splitting it is called indicase. Its action is as follows:

Plant indican. Indoxyl.

We may recall urine indican.



Urine indican. Indoxyl.

The indoxyl in each case then oxidizes itself by means of $O = O$ as already described (p. 92) to indigo blue.

Indigo is obtained by crushing the leaves of the indigo plant. Indoxyl is liberated by the indicase and changes to indigo blue. The chlorophyll is then dissolved out of the leaves by alcohol in which indigo blue is insoluble, and the indigo blue then dissolved out in chloroform to separate it from the cellulose skeleton of the leaf.

What the uses of glucosides are to a plant are somewhat obscure. The glucoside and its enzyme exist side by side in the cell, of a leaf for instance, and it is not until the leaf is bruised or dies that they come in contact and the reaction occurs. Since many of the products of the splitting are disagreeable to the taste it has been thought that these may serve as a warning to an animal which is eating a leaf, not to eat another, so that the storing up of the glucoside and its enzyme may be considered as a protective measure.

But in some cases the products are not disagreeable, to our taste at least, and certainly herbivorous animals will eat many glucoside-containing leaves with apparent satisfaction. It may be that, in some instances at any rate, this is a convenient way of storing glucose so that it is ready for an emergency. The leaf is injured, glucose is liberated by the enzyme, and the leaf uses it for building itself up again. The glucose is anchored to a cyclic compound to preserve it in stable form until needed.

Autolysis.

If a piece of animal tissue or organ is taken from the body aseptically and kept free from bacteria, decomposition, as it is ordinarily understood, does not take place.

If such aseptic tissue is heated up to 70° C. or 80° C. for an hour or so, it remains unchanged indefinitely, but otherwise it

undergoes changes which are not those of decomposition (bacterial action) but analogous to the digestive processes which go on in the intestinal tract.

It is gradually digested by the proteolytic enzymes contained in the cells. *Autodigestion* (*Autolysis*).

The products are the same in both instances, albumoses, peptones, and amino acids. Hydrolysis takes place but no oxidation. The peroxidases, by means of which oxidation may be partly carried on, are there, but are powerless to act because only O = O is offered them instead of their accustomed O = derived from HbO.

The cells of some organs also contain amylase and lipase, so that on autolysis the glycogen and fats if present are hydrolyzed to glucose and fatty acids.

CHAPTER VIII.

DISEASE AND IMMUNITY.

ALTHOUGH the subjects treated of in this chapter belong to pathology rather than physiology the two are so closely allied that a brief sketch may be attempted.

In a general way we may say,

Disease is caused by absorption of poison.

Immunity is defense against poison.

1. *Poisons* may be mineral or organic.

The organic poisons may be subdivided into:

1. Those secreted by plants, *e. g.*, vegetable alkaloids.
2. Those secreted by animals, *e. g.*, snake poisons.
3. Those secreted by intermediate forms of life, *e. g.*, bacterial poisons.
4. Waste or split products of any of these three forms of life, which may or may not be poisonous. These may be called *accidental poisons*.

They are not formed by the organism with a view to defense or attack.

PTOMAINS. TOXINS. LEUCOMAINS.

It is necessary to have a clear understanding of what is meant by these terms.

A. PTOMAINS.

Bacteria may be divided into two groups; the pathogenic, which can multiply in the body and cause disease, and the saprophytic, which are not able to multiply in the living body.

Decomposition of flesh is due to saprophytic bacteria and in the process they split up the proteid matter and furnish certain

soluble basic products, chiefly amins and oxyamins, called *Ptomains* (πτῶμα corpse). The ptomains are not necessarily poisonous but they may be and the danger of eating decomposing meat or fish is due to their possible presence. The ptomains belong to group 4.

B. TOXINS.

Toxins are synthetic products of bacteria.

They are not the split products of proteids but are substances synthesized by the bacteria inside the bacterial cell. When formed they may diffuse out of the cell or be retained within it.

As an example of each case we may take the bacilli of diphtheria and typhoid.

If a culture of diphtheria bacilli in broth is grown for two or three weeks and then filtered through a porcelain filter, the filtrate, although free from bacteria, is poisonous. It contains a soluble toxin excreted by the bacilli.

If a culture of typhoid bacilli in broth is grown for two or three weeks and then filtered through a porcelain filter, the filtrate is harmless. It contains no toxin.

But if the bacilli remaining on the filter are dried, thoroughly pulverized and suspended in water, the suspension is poisonous. The bacterial cells contain an intracellular toxin.

The courses of the two diseases also illustrate this. The diphtheria bacillus does not penetrate the tissues, but grows locally and superficially, forming a membrane on the surface of the throat for instance.

But from this point its soluble toxins are absorbed into the system and cause the symptoms of the disease. *Toxemia.*

Typhoid is a slow lingering disease. The bacilli get into the circulation and grow there. Period of invasion. After a time some begin to die off and, the bacteria disintegrating, the intra-

cellular toxins are liberated, and cause the fever. Later the bacilli gradually disappear from the blood, but the fever continues as long as any are left. *Bacteremia or Septicemia.*

C. Leucomains.

Leucomains are the products of the splitting of proteid by body cells. Leucomains are analogous to ptomains and may be identical.

For instance if lecithin is acted upon by bacteria, cholin may be split off. Cholin in this case is a ptomain. If lecithin is acted upon by a body cell, cholin may be split off. Cholin in this case is a leucomain.

Leucomains however do not leave the body as such in health. They undergo oxidation to urea, CO_2 and H_2O. But in certain pathological conditions, they may not be properly oxidized, and remaining in the system, act as poisons. *Autointoxication,* uremia for instance.

Immunity.

Immunity is the resistance of the body to poisons and its capability of ridding itself of toxic agents such as bacteria.

Immunity may be natural or acquired.

It takes a certain amount of any given poison to kill, and the smallest amount that will effect this is usually called the minimum lethal dose, M.L.D. Supposing a rabbit can stand an injection of .09 c.c. of diphtheria toxin, but that .1 c.c. will kill it, .1 c.c. is the M.L.D. and the rabbit has a natural immunity against .09 c.c.

A few days after a first injection of .09 c.c. into a rabbit a second dose may be given, and it now takes a much larger amount to kill; perhaps .2 c.c. By carefully raising the amounts the rabbit will soon be able to stand, say 1.0 c.c. or ten times the M.L.D., without being affected.

To its *natural immunity* against .09 c.c. an *acquired immunity* against .9 c.c. has been added. It is now immune to 1 c.c. or ten M.L.D. and is said to have been immunized to diphtheria toxin.

Antitoxins. — If some of the serum of this rabbit is injected into a second rabbit and at the same time a dose of diphtheria toxin, greater, even considerably greater, than the M.L.D. the second rabbit does not die.

The serum of the first rabbit contains something — *Antitoxin* — which protects the second rabbit.

The first rabbit has been *actively* immunized — with toxin. The second rabbit has been *passively* immunized — with antitoxin.

This is the basis on which the antitoxin treatment of diphtheria has been built up.

Just what antitoxin is, is still a matter of doubt, but since experiments have shown that toxin can be neutralized by antitoxin "in vitro," *i. e.*, in test-tubes in the laboratory, the neutralization clearly cannot be due to "vital action." Again the antitoxin is used up in the process so it is not a catalyzer, and its action is not analogous to that of enzymes.

There must, therefore, be a purely chemical combination between the toxin and antitoxin, and, taking this for granted, Ehrlich has constructed a theory which, for the present, serves very well as a working hypothesis.

Ehrlich's Theory of Antitoxins.

Every cell is built up of an innumerable number of proteid molecules, which can be divided into two groups.

1. The *central molecules*, which are concerned in cell metabolism.

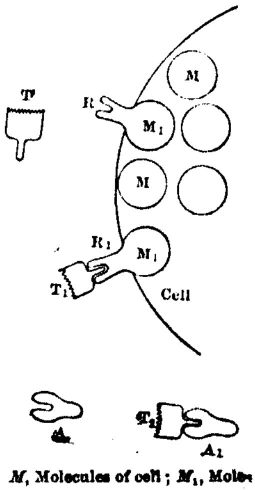
2. The *peripheral molecules*, which are concerned in cell nutrition.

The peripheral molecules are built up of a large number of *central nuclei*, amino acids, etc., in combination, and possess in addition side chains which are chemically active. We may consider benzoic acid $C_6H_5.COOH$ as analogous, the COOH being a chemically active side chain.

By means of their side chains the peripheral molecules are able to combine with side chains of various molecules passing along in the blood current and anchor them. *Analogy.*—Benzoic acid anchors glycocoll to form hippuric acid. Such anchored molecules may be passed on to the central molecules and utilized by them.

A toxin molecule is anchored in the same way, but instead of being an assistance it paralyzes the side chain and throws it out of action. If many of the side chains are thus thrown out of action the cell dies, and if a sufficient number of cells are thrown out of action, the entire organism dies.

DIAGRAM I.



M, Molecules of cell; *M*1, Molecules with receptors; *R*, Receptor; *R*1, Receptor with toxin; *T*, Toxin; *T*1, Toxin with receptor; *T*2, with antitoxin; *A*, Antitoxin; *A*1, Antitoxin occupied by toxin.

The toxin has killed the animal.

But the amount of toxin anchored may only be sufficient to injure the cell temporarily, so that it can recover. It is a well-known fact that injury is followed by hypertrophy, *i. e.*, overproduction of the tissues locally at the point of injury.

For Example. — A callus is formed as a broken leg heals.

Just so with the injured cells. There is over-production at the site of injury, *i. e.*, the side chains of the peripheral molecules (usually called cell receptors). These

may be produced in great excess, and a number of them become detached and float away in the blood current. These cast-off side chains or receptors are the *antitoxins*.

They have now acquired specific properties, *i. e.*, will anchor the particular toxin which caused their production but nothing else.

Additional amounts of this particular toxin now introduced into the circulation, do not reach the cells, but are anchored and rendered inert by the antitoxins in the blood current.

The cells, therefore, escape injury. (Diagram 1.)

The success of the antitoxin treatment of diphtheria led to hopes being entertained that all bacterial diseases could be equally well overcome by means of antitoxins.

This, however, is not the case. Tetanus and diphtheria kill by *toxemia* and the body cells form antitoxins to resist this, but most pathogenic bacteria cause disease and death by *bacteremia* and the immunization process against this is somewhat different. Antitoxins are not formed in bacteremia or only to a limited extent; not sufficient for the blood to protect other animals passively.

Immunization Against Bacteremia.

The typhoid bacillus can be taken again as an example.

Agglutinins.

The blood serum of a patient with typhoid fever acquires the property of agglutinating typhoid bacilli. Observed in a hanging drop under the microscope the bacilli are seen to be very motile. If a drop of diluted normal blood serum is added, the motility is not affected, but on adding a drop of diluted serum of a typhoid patient the bacilli lose their motility, and collect together in clumps.

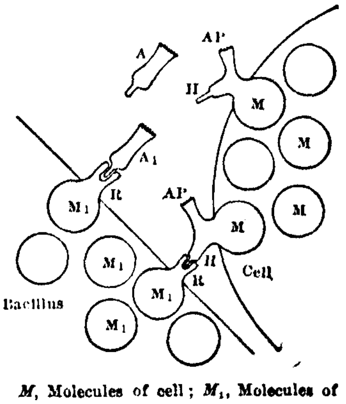
The clumping can also be observed macroscopically in a test-tube. If to 1 c.c. of a typhoid culture in broth 1 c.c. of typhoid serum, diluted say to 1–25 with physiological salt solution, is added, making the total dilution 1–50, the mixture soon appears flaky, small clumps become visible which gradually sink to the bottom of the test-tube, leaving the fluid above perfectly clear.

This is called the clump or Widal reaction and is very generally employed for diagnosis of typhoid fever.

The serum of rabbits or other animals inoculated with cultures of typhoid bacilli also acquires agglutinating properties. By repeated injections the serum can be made very active, so that dilutions of 1–20,000 or even higher may show the reaction.

It appears that under the influence of the immune serum a sticky substance is exuded from the bacilli. This is called the *agglutinum;* and the active substance in the serum, the *agglutinin.*

DIAGRAM II.



M, Molecules of cell; M_1, Molecules of Bacillus; *AP*, Agglutinophore Group; *H*, Haptophore Group; *R*, Receptors occupied by agglutinins; *A*, Free Agglutinin; A_1, Agglutinin with Receptor.

The agglutination, however, does not kill the bacilli. It only makes them somewhat inert, so that in the presence of agglutinins they cannot multiply or only very slowly.

Ehrlich's theory with regard to this is that some of the side chains of the peripheral molecules of a body cell are supplied with two groups, a haptophore (seizing), and a toxophore (toxic) group. (Diagram II.)

The haptophore group H seizes a receptor R of the bacillus and the toxophore or agglutinophore group AP influences the bacillus in such a way that it exudes the agglutinum.

If the body is invaded by the bacilli so many of these particular side chains are thrown out of action that the cell is injured to this extent. There is therefore over-production, and many of the new side chains are cast off into the circulation where they occur as free agglutinins. The agglutinins are specific. They will agglutinate the bacterium of invasion but no other.

PRECIPITINS.

A similar curious phenomenon has also been observed.

If a broth culture of typhoid three or four weeks old is filtered through porcelain and thus freed from bacilli, the clear filtrate, if immune serum is added, will shortly become flaky, with formation of clumps which gradually sink to the bottom of the test-tube.

This is the precipitin reaction. The active substance in the serum is called the *precipitin* and the passive substance in the filtrate which is precipitated is called the *precipitum*.

The serum in this case must be much more concentrated than for agglutination. A serum which will agglutinate typhoid bacilli in dilution of 1–10,000 will probably not cause precipitation in a filtrate in higher dilutions than 1–20 or 1–40.

Filtrates of fresh cultures cannot be precipitated in this way.

The precipitum is probably an albumenous substance liberated on the death of the bacilli, and is no doubt closely allied to the agglutinum though it is still an open question if the two are identical or not.

LYSINS.

The agglutinins, as already mentioned, do not kill but only hold the bacteria in check, yet the bacteria must be killed in order to get rid of them.

Normal serum if fresh will kill a few bacteria, and this power is greatly increased against any particular bacterium on immunization with it. This increased power can be more clearly demonstrated "in vivo" than "in vitro," by means of what is known as Pfeiffer's phenomenon. Pfeiffer experimented with cholera bacilli but the same thing applies to typhoid.

If a large number of typhoid bacilli are injected into the peritoneal cavity of a normal guinea-pig (or rabbit, etc.), and a small quantity of the peritoneal fluid withdrawn from time to time by means of a capillary glass tube, microscopical examination shows that the bacilli after two or three hours are still numerous and actively motile. There are more of them than the normal fluid can kill and in all probability they will multiply and set up a fatal bacteremia.

If the same thing is done with a guinea-pig immunized to typhoid the bacilli immediately lose their motility and clump. Before long they become granular and begin to break up. Cultures taken from time to time show that the bacilli are killed within a few hours. The agents which kill them are called *bacteriolysins.*

An analogous case is that of the *hemolysins*, *i. e.*, agents which destroy red blood corpuscles, and since the action of these is much more easily demonstrated "in vitro" than the action of bacteriolysins it is chiefly on experiments with hemolysins that Ehrlich has built up his theory of the lysins.

We can take two animals, the goat and the rabbit, as examples, but what applies to them applies to other animals also.

Normal rabbit serum can dissolve the red corpuscles of a goat to a very limited extent in a test-tube, but this power is enhanced to a remarkable degree if the rabbit is treated with successive injections of goat's blood. The serum of the rabbit has become strongly *hemolytic* for goat's corpuscles.

As an example of an experiment we may dilute 1 c.c. of hemolytic serum with 99 c.c. physiological salt solution; take 1 c.c. of the dilution in a test-tube and into this put one drop of defibrinated goat's blood. As the red corpuscles dissolve the hemoglobin diffuses out and the fluid becomes red. To see if there has been total hemolysis or not a microscopical examination can be made.

If such hemolytic serum is warmed to 60° C. for 30 minutes it will no longer destroy goat corpuscles. It has become *inactivated.*

Supposing we now take normal rabbit serum and add a few drops of it to the fluid. The red cells are quickly dissolved. The *inactivated* serum has been *reactivated* by normal serum. Why does normal serum have this power? We shall see directly.

With bacteriolysins there is no simple method of observation like this, as bacteria have to be added to the serum, a loopful of the fluid plated out at intervals and the colonies counted; a tedious and not very accurate method.

But the bacteriolysins act in just the same way as hemolysins, so we can now return to our typhoid bacilli to work out the meaning of these phenomena.

Ehrlich's theory extended to the lysins, supposes that some of the peripheral molecules of the body cells contain side chains specially constructed to grapple with bacteria, and probably other inert foreign bodies and destroy them. It must be remembered that the bacteria themselves appear to the body cell simply as inert foreign substances. It is only their toxins which actually cause direct injury.

Such special side chains contain two groups, a chemically active haptophore group, and a latent receptor group.

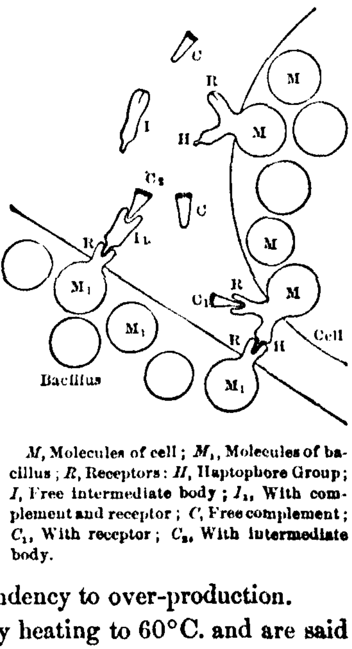
The haptophore group can seize a receptor of a bacillus and

anchor it, but cannot of itself exert any influence on the bacillus. But as soon as it has anchored the bacillus its latent receptor group becomes chemically active and capable of receiving a body which is normally present in the blood called a *complement* (also called alexine or addiment).

The combined action of the complement and special side chain can destroy the bacillus.

But although the cell is not directly injured these special side chains are thrown out of action, and consequently overproduction takes place: Many are cast off and circulate free in the blood current. They are now known as "*immune*" or "*intermediate*" bodies or *amboceptors*, and with the aid of the complements destroy the bacilli.

The intermediate bodies like the antitoxins and agglutinins are produced in excess in immunization, but the complements do not increase in number. They exist normally in the body and the cells, — *i. e.*, the leucocytes,—which produce them are not in any way affected by the bacteria so there is no tendency to over-production.



M, Molecules of cell; M_1, Molecules of bacillus; *R*, Receptors: *H*, Haptophore Group; *I*, Free intermediate body; I_1, With complement and receptor; *C*, Free complement; C_1, With receptor; C_2, With intermediate body.

Complements are destroyed by heating to 60°C. and are said to be thermolabile, whilst the intermediate bodies are only affected at considerably higher temperatures, 70–75°C., so are called *thermostable*.

We can now understand why immune serum is inactivated

by heating to 60°C. (Complements are destroyed, but not intermediate bodies), and can be reactivated by adding normal serum. (Intermediate bodies supplied with fresh complements.)

Again complements once separated from the body quickly break up and disappear from the serum, whereas intermediate bodies remain intact for months. For this reason serum used in experiments with lysins must be fresh, not more than one or two days old. But as with heated serum old inactive immune serum can be reactivated with fresh normal serum. Pfeiffer's phenomenon does not show with a normal animal because there are only complements present in the peritoneal fluid, whilst with an immunized animal both bodies exist free in the peritoneal cavity.

The intermediate bodies are *specific*. They will only attack the particular species of bacterium or blood cell against which the animal has been immunized, whilst the complements are *general*, being able to combine with any intermediate body, although it may be that bacterial complements are not the same as hemolytic complements.

It must be confessed that this has been put in a very general way. There are numerous partial exceptions to the rules here laid down and numbers of cases which do not seem to conform to them at all. The consequence of this is that Ehrlich has been obliged so to extend and complicate his theory to meet all the requirements, that it is becoming doubtful if it will stand the strain much longer. Some new and more simple theory for the phenomena of bacteremia is urgently needed but as yet there is none in sight, and for the present we must make the best we can of Ehrlich's.

A study of the reactions against bacteremia and the theories to account for them makes one curious to know what is the meaning of the double and apparently independent action of the

agglutinins and bacteriolysins. We can only guess, but it seems quite possible that agglutination is a means of holding the bacteria in check until they can be destroyed.

On introducing bacteria into the circulation some are at once destroyed by the cell side chains and complements, and as soon as intermediate bodies are formed still more can be destroyed. But the complements are soon used up and it is necessary to wait for a fresh supply. Meanwhile side by side with the intermediate bodies free agglutinins have been produced and these help to prevent multiplication until more complements are ready.

The question then arises: "Why is not some provision made for a larger supply of complements so that the bacteria may be destroyed more quickly?" The answer seems to be, that it would not do to kill the bacteria off too quickly. It must be remembered that they contain intracellular toxins which are only set free on the death of the bacilli and against these intracellular toxins antitoxins do not appear to be formed or at any rate only to a limited extent, so that the liberation of large amounts of toxins all at once would be dangerous and liable to cause the death of the organism.

True in Pfeiffer's phenomenon a large number of bacteria can certainly be killed in a short time, yet there is always a limit to the number, depending on the number of complements present. We are dealing here too with highly immunized animals which can no doubt stand a large dose of toxin even though there may be little or no antitoxin in the blood.

It is quite possible that, instead of the organism as a whole becoming immunized to the intracellular toxin by means of antitoxin in the circulation, the individual cells gradually get hardened to it.

Analogous cases can be found in mineral poisons. An arsenic

eater can take enough arsenic in 24 hours to kill a dozen ordinary people, yet there are no antiarsenic bodies in his blood. One can only suppose that the individual cells have accustomed themselves to the poison in some way, so that they are no longer injured.

It may be said that no antibodies have ever been experimentally produced in serums against poisons of a relatively simple nature whose chemical constitutions are known.

In order to avoid misunderstanding it may be mentioned that a number of workers claim to have succeeded in obtaining antityphoid serums, the use of which is beneficial in cases of typhoid fever. But these serums have never come into general use and the tendency is to discredit them. The same may be said of antistreptococcus and antipneumococcus serums.

On the other hand recent experiments by Vaughan indicate that the toxins of typhoid and intracellular toxins in general are relatively simple bodies, against which one would not expect antitoxins to be raised.

By breaking down the bacterial cells with sulphuric acid he has extracted virulent toxins which resist boiling. Highly complex molecules would certainly be destroyed under these conditions.

Before closing the chapter a further reference to the precipitins must be made.

It appears that any albumenous substance when injected into an animal gives rise to precipitins in the serum, *i. e.*, "antibodies" which will precipitate it from solution, and moreover the anti-body is always specific for that particular albumen. In this way a large number of albumens which had always been supposed to be precisely alike have been shown to differ to some infinitesimal extent, a difference which cannot be detected by their ordinary chemical or physical (*e. g.*, salting out) reactions.

Differentiation of albumens by this means is called the *biological* test.

Examples: 1. If the milk of a cow is injected into a rabbit, the serum of the latter will precipitate the casein of cow's milk, but not that of human or other milk, or only to a very slight extent.

2. If the blood of a cow or ox (beef blood) is injected into a rabbit the serum of the latter will not only contain hemolysins but also precipitins, so that on adding a few drops of the immune rabbit serum to 1 c.c. clear beef serum, the latter becomes flaky with precipitation of clumps to the bottom of the test-tube.

This reaction is made use of as a test for human blood.

The serum of a rabbit immunized to human blood (humanized rabbit) will cause a precipitate in diluted human serum or even in an aqueous solution of blood which has been dried out for months, so that by this means it can be determined if blood stains are those of an animal or human being.

The precipitin is specific though not absolutely so. A humanized serum which will precipitate human serum at 1–100 may precipitate that of an ape at 1–30, and that of a dog at 1–10 for example.

The more nearly related the animal the more likely is its blood to react. There is probably strict specificity for the particular albumen, but other kinds of blood may contain small proportions of it.

We may suppose that human blood contains of albumen A 50 per cent. and of B 50 per cent., both of which give rise to precipitins. The blood of an ape may have 10 per cent. of A, 20 per cent. of B and 70 per cent. of C, whilst that of a dog has 10 per cent. of A, 10 per cent. of C and 80 per cent. of D.

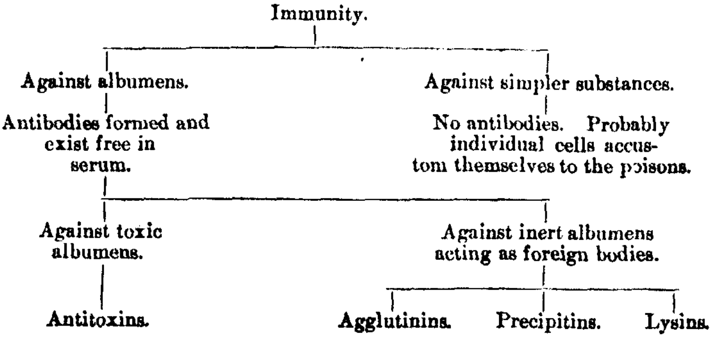
If three rabbits are immunized, each with one kind of blood

to such an extent that the serum of each reacts at 1–100 with its blood of immunization we would have:

	Human Serum.	Ape Serum.	Dog Serum.
Rabbit No. 1 with human blood	1–100	1–30	1–10
" " 2 " ape "	1–30	1–100	1–20
" " 3 " dog "	1–10	1–20	1–100

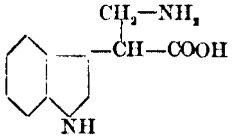
We have throughout said that the agglutinins and lysins are specific, but they are not absolutely so any more than the precipitins. It has therefore been suggested that all these reactions should be called *special* rather than specific, but there seems no good reason for this. They are specific enough for all practical purposes so may just as well be designated as such.

Probably all anti-bodies, not only precipitins, but also lysins and agglutinins are formed, not against particular cells, but against particular albumens contained in those cells. Thus a rabbit serum which will agglutinate the typhoid bacillus 1–20,000 may agglutinate the Bacillus coli communis 1–100 or 1–200. This simply means that Bacillus coli communis contains a small proportion of the albumens which go to make up the protoplasm of the typhoid bacillus.

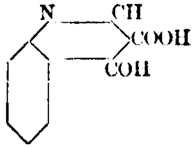


POSTSCRIPT.

While this book was going through the press a new formula for tryptophan was suggested by Ellinger which explains very well the relations existing between this substance and kynurenic acid.



Tryptophan.



Kynurenic acid.

When tryptophan is fed to a dog kynurenic acid is excreted. If gelatin, which does not give the Adamkiewicz reaction and therefore does not contain the tryptophan group, is fed kynurenic acid is not formed. The new formula for tryptophan is probably the correct one but it does not affect the comparison made between the products of tyrosin and tryptophan on p. 128.

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